The treatment of irritable bowel syndrome using a novel multi-strain probiotic

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Thesis for award of: MD(Res)

Acknowledgments.

I would like to thank Professor Ingvar Bjarnason whose continued unwavering support, guidance and friendship have been essential in the production of this thesis. I would also like to thank Inge Bjarnason for the particular contributions he made during this research study. Lastly, I would like to thank my partner for their support, encouragement and acceptance during what have not always been the easiest of times. I thank you all individually and together because without you this would still be sitting in pieces incomplete.

Funding.

This research project was funded by Kings College Hospital via and unrestricted research grant from Symprove Ltd.

Abstract.

Background

The importance of interactions between the host and gut microbiota in the pathogenesis of irritable bowel syndrome (IBS) is becoming increasingly apparent. Probiotics offer a potential new therapy for the treatment of IBS, but current results with these products are conflicting, largely as a result of poorly designed trials and non-standardisation of outcome measures.

Aims

The objective of this study was to assess the efficacy and safety of a liquid, multi-strain probiotic in the treatment of IBS.

Methods

A single-centre, randomised, double-blind, placebo-controlled trial of adult patients with symptomatic IBS. Patients received 12 weeks of treatment with the probiotic or placebo (1ml/kg/day). The primary efficacy measure was a change in the IBS symptom severity score (IBS-SSS) from baseline to week 12. A secondary outcome measure was a change in the IBS quality of life (IBS-QOL) score.

Results

A total of 186 patients were randomised and 152 patients completed the study. The mean difference in change in IBS symptom severity scores between the two groups was statistically significant (-35.0 (95% CI; -62.03, -7.87); p=0.01). Adverse events were mild and transient and no serious adverse events were reported.

Conclusion

The multi-strain probiotic is associated with an improvement in overall symptom severity in patients with IBS, is well tolerated and has a good safety profile.

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List of abbreviations:

5-HT	5-hydroxytryptamine.
ALP	alkaline phosphatase.
ALT	Alanine transaminase.
ATP	Adenosine triphosphate.
CBT	Cognitive behavioural therapy.
CI	confidence interval.
C-IBS/IBS-C	Constipation predominant irritable bowel syndrome.
CNS	Central nerve system.
CRF	Corticotrophin releasing factor.
CRP	C-reactive protein.
D-IBS/IBS-D	Diarrhoea predominant irritable bowel syndrome.
Ec	Enteroendocrine.
EMEA	European agency for the evaluation of medicines.
ENS	Enteric nervous system
ESR	Erythrocyte sedimentation rate.
FAE	Follicle associated epithelium.
FBC	Full blood count.
FDA	Federal Drug Authority.
FGID	Functional gastrointestinal disorders.
FODMAP	Fermentable oligo- di and monosaccharaides and polyols.
GABA	Gaba-aminobutyric acid.
GALT	Gut associated lymphoid tissue.
G-GT	gamma-glutamyl transpeptidase.
GI	Gastrointestinal.
HLA (DQ2)	Human leukocyte antigen.
IBD	Inflammatory bowel disease.
IBS	Irritable bowel syndrome.
IBS-QOL	IBS quality of life score.
IBS-SSS	IBS symptom severity score.
Ig	Immunoglobulin (IgA, IgE, IgG)
IL	Interlukin (IL-10).
ISRCTN	International standard randomised controlled trial number.
ITT	Intention to Treat
LBT	Lactose breath test.
LFTs	Liver function tests.
LIV	Linde internal validity scale.
LOCF	Last observation carried forward.
M- IBS/IBS-M	Mixed irritable bowel syndrome.
MALT	Membrane associated lymphoid tissue.
MCID	Minimal clinically important difference.
MRI	Magnetic resonance imaging.
mRNA	Messenger ribonucleic acid.

NAFLD	Non-alcoholic fatty liver disease.
NDMA	N-methyl-D-aspartate.
NNT	Number needed to treat.
NRES	National research ethics service.
OR	Odds Ratio.
PAR	Protein activated receptor.
PGE2	Prostaglandin E2.
PI-IBS	Post-infectious irritable bowel syndrome.
PP	per-protocol
QOL	Quality of life.
RCT	Randomised control trial.
rDNA	Ribosomal deoxyribonucleic acid.
RR	Relative Risk.
rRNA	Ribosomal ribonucleic acid.
SBPS	Small bowel permeability study.
SCFA	Short change fatty acids.
SD	Standard deviation.
SIBO	Small intestinal bacterial overgrowth.
SMD	Standardised mean difference.
SSRI	Selective serotonin reuptake inhibitor.
TNFα	Tumour necrosis factor alpha.
TTG	Tissue transglutaminase.
U-IBS/IBS-U	Unclassified irritable bowel syndrome.

Chapter 1:

Introduction

1.1 Introduction

Irritable bowel syndrome (IBS) is a common diagnosis given to patients with a variety of gastrointestinal symptoms and is one of the major recognised functional gastro-intestinal disorders. IBS is the term to describe or categorise a cluster of relapsing and remitting gastrointestinal symptoms that encompass a variety of interrelated heterogenic conditions grouped together under one 'umbrella' term. It is this heterogenic clustering of symptoms, including diffuse or localised abdominal pain often relieved by defecation, abdominal bloating, change in bowel openings, tiredness and passing of mucus amongst others; coupled with absence of conventional organic disease that essentially characterises the condition and makes the diagnosis clinically. Although often thought of as a 'modern disease' it was first noted in 1871, in The American Journal of Medical Sciences by JM Da Costa who first described 'a painful affliction of the colon, membranous enteritis, a condition which is but very incompletely known and scarcely recognized as a separate disease by the profession'.[1] It was several decades until the terms 'spastic colon' or 'the irritable colon syndrome' were widely used or recognised; but Da Costa's work is frequently cited by later authors as one of the first descriptive scripts of the condition that is now commonly referred to as IBS.

In 1931, Edmund Spriggs in his substantial work ‘functional disorders of the colon’ gives a full description of ‘the functional disorder of the colon’ akin to IBS. Interestingly, in this work he alludes to several aetiological and pathogenic concepts in IBS namely; the association of the disorder with the aftermath of infective tropical dysentery, pyorrhoea and other septic infections of the mouth and throat, the acquirement or inheritance of an ‘unstable nervous system’ as a predisposing risk factor and the response of symptoms to a simpler diet than the ‘modern civilised diet [2] and other concepts that remain a significant focus in research literature and study today. Later, in 1962, Chaudhary and Truelove further described the ‘irritable colon syndrome in an authoritative and influential publication. They included the recognition of possible sub-groups according to bowel habit (constipation or diarrhoeal predominance) and the apparent efficacy of early treatment with phenobarbitone or codeine.[3]

The features of IBS often differ markedly between patients both in terms of abdominal symptoms and severity, bowel habit and associated extra-intestinal symptoms. Response to treatment and natural history of the disease often varies markedly and as such it is difficult to consider IBS as a single entity. Several attempts have been made to develop a standard of nomenclature and diagnosis for all functional gastro-intestinal disorders including IBS. The most recent and widely used of these is the so called ROME III (table 1) criteria, [4] which is a set of diagnostic criteria based on

international consensus opinion of IBS researchers in an attempt to standardise clinical trials and practice to allow for a meaningful comparison of studies. The ROME III criteria sub divides IBS according to the predominant pattern of bowel habit into constipation (C-IBS), diarrhoea (D-IBS), both diarrhoea and constipation – mixed (M-IBS) and unclassified (U-IBS) where there is little or no change in bowel habit (table 2).[5] Whilst it is not necessarily the convention, it may be useful in both research and clinical practice to consider IBS as a group of interrelated but different disorders that share the common symptom of abdominal discomfort/pain. Differing aetiology, prevalence, treatment strategies and responses of the individual sub types should also not be overlooked, but unfortunately high quality data on these aspects of the disease is often lacking.

Today patients frequently consult their physician and gastroenterologist with a variety of abdominal symptoms akin to IBS. However, over a century since its first description and despite significant advances in our understanding of the epidemiology and pathogenesis of IBS the availability of efficacious treatments remains extremely limited.

1.2 The epidemiology of irritable bowel syndrome.

Many studies can be found in the literature that estimate the prevalence of IBS in differing communities, with estimates ranging from 2.5% -22%.[6-34] These studies are summarised in table 3. The reasons for the significant differences in prevalence estimates are numerous and complex. One of the principle reasons is the heterogenic nature of the condition and the lack of a definitive diagnostic test. As such clinicians and research groups are reliant on a combination of negative tests to exclude other organic diagnoses and the application of certain diagnostic criteria to make a considered if not definitive diagnosis of IBS.

There has been much evolution in the diagnostic criteria since the original Manning criteria were first published in 1978.[35] The original Manning criteria comprised a simple list of symptoms in keeping with a diagnosis of IBS (table 1). The presence of greater than two, greater than three or greater than four symptoms from this list gave rise to the 'Manning 2', 'Manning 3' and 'Manning 4' criteria respectively. In the study by Talley et al. in 1991 a postal survey of 1021 members of the general population in America, 835 respondents reported a prevalence of 17.0%, 12.8% and 8.7% using the Manning 2 ,3 and 4 criteria respectively.[13] Jones et al. 1992, in a similar postal survey of 2280 (1620 respondents) general practice patients in

the United Kingdom, using the Manning 3 criteria reported a somewhat higher prevalence of 22%. Whilst both of the studies utilised a postal survey for data collection they were looking at different populations (USA and UK). In addition, Jones et al. used a single questionnaire designed by the study group based on previous published works whereas Talley et al. garnered the information from a general health questionnaire. Neither questionnaire was validated specifically to identify patients with IBS although Jones et al. amalgamated questions from several previously published, validated questionnaires. The Jones et al. study is perhaps more likely to suffer from a response bias as it was aimed at identifying a particular condition from within the target population and hence subjects with the condition may be more likely to respond.[9] Further studies using the Manning criteria return prevalence estimates of between 8.7% and 22%.[8, 9, 13, 17, 18, 22] The Manning criteria are the least specific of the criteria and as such give rise to the highest prevalence estimates and are likely to overestimate the true prevalence of the condition.

The Manning criteria were later superseded by the Rome criteria in 1989. The concept for the Rome criteria was first discussed at the 12th International Congress of Gastroenterology in Lisbon 1984 due to the growing need for a standardised, robust set of diagnostic criteria that could be used comparatively in both research and clinical practice. Subsequently, the Rome Guidelines, agreed at the next congress in Rome in 1988, were

first published in 1989.[36] Since this time the Rome criteria have undergone several reviews and revisions and now include a full classification based on international consensus opinion of experts in the field, of all the functional gastrointestinal disorders including IBS. This has led to several criteria namely; the Rome (1989) [36], Rome 1990, first revision [37], Rome I (1994) [38], Rome II (1999) [39], and the latest Rome criteria, The Rome III (2006). [5] The Rome criteria apply more specific and stringent diagnostic criteria to the diagnosis of IBS as well as including a temporal component (Table 2). These more stringent criteria have ultimately led to lower prevalence estimates than given in studies using the Manning criteria.

Several authors have compared the differing diagnostic criteria on the same population. Hahn et al. (1997) compared Manning 2 and Rome (1989) using data from 42,392 face-to-face interviews of the National Health interview survey (NHIS) in America. They reported a prevalence of 8% and 3% respectively.[17] The Rome criteria returns a much lower prevalence than the Manning criteria, which is consistent with other studies although the overall prevalence in this study was significantly lower than the majority of studies in similar populations. This disparity may be a result of the direct face-to-face interviews used in the study. Mearin et al. in 2001 also used face-to-face interviews of 2000 subjects in Spain to compare the Manning and Rome I and Rome II criteria. This study unusually, reported a higher incidence with Rome I than the Manning criteria; 12.1% compared to 10.3%

but went on to report a markedly lower prevalence of 3.3% when the Rome II criteria were applied.[11] Saito et al. in (2000) in a study of 892 subjects in a postal survey of the American general population, reported a prevalence of 20.4%, 15,7% and 13.1% for the Manning 2, Manning 3 and Rome criteria respectively.[18] These results are similar in scale to those shown by both Talley et al. (1991) and Jones et al. (1992).[9, 13]

Later studies using the Rome II standard consistently returned a lower estimate of prevalence when applying the Rome II criteria compared to any of the preceding criteria. Saito et al. (2003) is an example of this, in their study that compared 892 (643 respondents) subjects of the general population in the USA via a postal survey using Rome (1989), Rome (1990), Rome I and Rome II and finds a prevalence of 27.6%, 5.1%, 6.8%, and 5.1% respectively.[12] One exception to this is Thompson et al who in 2002 reported a higher prevalence of 13.1% with Rome II compared to 10.3% with Rome I criteria. The most recent Rome III criteria were first published in 2006 and to date their use for prevalence studies has been limited to populations other than the USA or UK. As such it is not possible to confidently state how the prevalence estimates in these populations would be affected by the application of the new criteria. Studies using the Rome II criteria have been performed but in differing populations. The reported prevalence using Rome III in these populations is 7.85% (2126 ,North China university students)[28], 19.4% (2196 German university students) [31] and 4% (4767 subjects from

general population of India).[33] We found only one study that directly compared the Rome III criteria with previous classifications. This paper, conducted by Sperber et al. compared the Rome II and Rome III criteria in a sample of 1221 subjects (1000 respondents) of the general population of Israel and reported a prevalence of 2.9% and 11.4 % respectively.[34]

It is clear that prevalence estimates vary dramatically between different studies and populations. As previously stated the heterogenic nature of the condition, differences in cultural expectations and the lack of a specific diagnostic test contribute significantly to this. The application of differing diagnostic classification systems also has a dramatic impact upon the reported prevalence estimates. On one side, the Manning criteria is likely to overestimate the true prevalence by being too non-specific and not applying a temporal component to the criteria. On the other side, the Rome II and III criteria undoubtedly exclude a significant amount of IBS at least in part from the application of strict temporal and frequency criteria. What is not apparent from the published studies is why the comparative data when applying the different classification criteria does not result in differences of a similar magnitude. For example, Mearin et al. (2001) compared Rome I and Rome II; finding a difference between the two criteria in the order of more than three times lower prevalence with the older criteria (12.1% and 3.3%). Saito et al. (2003) however, compared the same criteria and found little difference between the estimated prevalence (6.8% and 5.1%).[11, 12] The

implication from this is simple, as although the criteria themselves may be robust their application in prevalence studies is open to individual interpretation resulting in quite different results between study groups. The latest Rome criteria (Rome III) are too restrictive and as a result may actually exclude a substantial number of patients that experienced clinicians would likely accept as having a clinical diagnosis of IBS. This may be of no significant consequence to clinical practice but in the research environment may substantially bias the results of clinical trials by excluding patients who do have IBS. Given the significant heterogeneity of IBS and the current lack of established biological markers it is unlikely that any diagnostic criteria will be completely accurate. However, a balance between usability and application both in research and clinical practice are essential if criteria are to be widely adopted. The Rome IV criteria, when agreed and published will hopefully go a step further to addressing some of these issues.

Differing methodological approaches using retrospective patient data, medical insurance data and diagnostic coding or prospective data with postal survey, face-to face interviews, telephone interviews and self-reporting symptom questionnaires are also likely to have a significant influence on the estimated prevalence. The specific influence these factors may have is likely to be complex and not enough data is available in the literature to give a considered opinion. Caution should therefore always be exercised when comparing studies with different collection and sampling methodologies. IBS

symptoms themselves are also often transient in nature and as such the phrasing of questions that ask ‘have you ever’ or ‘are you currently’ suffering from will clearly affect the results. One such study looked at this transient characteristic of symptoms and found that 38% of the study cohort who reported IBS symptoms did not meet diagnostic criteria in a self-reported questionnaire one year later.[40]

The majority of epidemiological report a female predominance of IBS although gender differences show considerable variability ranging from as high as 1.0: 4.3 (male: female) to equal prevalence in some studies (table 3). The majority of studies however report ratios in the order of 1:1 to 2:1 (male : female) in studies of western populations.[41] India appears to differ with a male predominance of 4.2:1.0 in young adults.[42] The overall trend in the incidence of IBS is that it decreases with increasing age; 4.2% of the age group 30-39 compared to 2.7% in those aged 60-69 in one study (overall prevalence 4.7%).[12] Symptomology may also change with gender and age with constipation being more prevalent in females and older individuals.[13, 43] There is also a marked difference in the reported prevalence between different populations. The incidence seems to be generally higher in America, Canada, USA and Europe with intermediate rates in eastern countries such as China, Korea and Singapore and the lowest rates in developing countries like India. The reasons for these differences, in addition to methodological considerations, may include both environmental and

genetic factors as well as differing urbanisation, socioeconomic and health care.[44-46]

There is no consensus opinion on the prevalence of IBS for any specific population. In developed countries it is likely to be in the order of around 7-10% of the general population and may be significantly higher. Regardless of the particular diagnostic criteria, IBS is associated with significantly increased healthcare costs [47-51] and absenteeism.[52] However, of those people who report symptoms that meet criteria for IBS only 9%-33% consult their physician about their symptoms.[9, 51] Healthcare costs for patients with IBS were significantly higher (by a factor of 1.1-6.0) when compared with non-matched non-IBS controls in two studies; one in America and one in the UK.[52, 53] In addition to the diagnostic and therapeutic costs of treating IBS, the indirect healthcare costs are also significantly higher.[54] In trying to rationalise and understand these costs, research has focused on understanding the characteristics or circumstances which result in an IBS patient consulting their physician or accessing healthcare in other ways.

It is widely reported in the literature, and a commonly held belief amongst clinicians and specialists, that patients with IBS have a higher incidence of co-morbid psychological disease and that this is a fundamental

component in the aetiology of IBS symptoms.[55-64] In fact, Chaudhury and Truelove (1962) report that 80% of their 130 cases had contributing psychological factors. These factors consisted of diagnosable psychiatric illnesses such as depression, personality traits such as increased anxiety and environmental stress such as marital or family problems.[3] A retrospective review of this early work has resulted in the opinion that inappropriate methodology may have led to an overestimation of the prevalence of psychiatric illness in IBS and in my opinion this is probably inappropriately quoted as evidence for its role in the aetiology of the condition.[55] The role of psychological factors in the aetiology and management of IBS is discussed in more detail later and needs to be viewed in the different context of aggravating the condition.

It is important to consider the influence that the methodological factors have on the reported prevalence of the disease. The methodologies used for data collection in the epidemiological studies for IBS vary greatly with many relying on self-reported questionnaires about abdominal symptoms. Such questionnaires will undoubtedly be influenced by any psychological factors affecting the cohort studied. The environment from which the data is collected may further compound this effect. For example, IBS cases can be over represented within the patient group; this is likely to be the case in a primary care setting as IBS sufferers are known to be more frequent users of these healthcare services. Accordingly, the result will only be relevant to that

cohort and should not be extrapolated as representative of IBS cohorts in general. [65] Psychological factors may also introduce response bias into large scale population studies, as cases with underlying psychological problems may be more or less likely to respond depending on their state of mind.

In Summary, the heterogenic nature of IBS, associated psychological factors, differing classifications and methodology and the lack of a specific diagnostic test has undoubtedly led to significant variation and inaccuracies. Consequently any reported prevalence must be considered with caution.

1.3 The aetiology of irritable bowel syndrome

To date the understanding of the aetiology of IBS is limited and whilst in recent years there have been significant advances in attempts to understand the underlying possible pathogenic mechanisms of IBS, there is still no single accepted theoretical model of IBS. If we accept the idea that IBS is not likely to be a single entity, but to consist of a group of inter-related but differing conditions with similar symptomology, then logically we should also expect differing pathogenic / pathophysiologic mechanisms and aetiologies. IBS is likely to be the result of a complex interaction of a multitude of different environmental and host components, including both

physical and psychosocial elements and principles.

1.3.1 Post infectious irritable bowel syndrome

Post-infectious irritable bowel syndrome (PI-IBS) is the term used to define a sub-group of patients, where there is a clear temporal relationship between an acute gastro-intestinal infection and the subsequent persistence of symptoms akin to IBS. PI-IBS tends to predominantly but not exclusively lead to IBS-D. Chaudhary and Truelove reported that 25% of their patient group had a clear episode of infective gastroenteritis prior to developing IBS; but do not give further details of the temporal relationship between the infective insult and the development of symptoms.[3] Since this work, multiple retrospective and prospective studies have re-examined the possible link between enteric infection and the development of IBS.

A retrospective study of 124 patients and 120 controls reported an increased incidence of PI-IBS at three years post-infection with a strain of *Shigella sp.* (14.9%) compared to controls (4.5%) with an odds ratio of 3.93. They also showed that the recovery rate for PI-IBS was lower in patients with a previous history of functional bowel disorders other than IBS.[66] Similarly, a prospective study of 72 patients with confirmed *Trichinella Britovi* infection during an outbreak in Turkey, had a significantly higher rate of IBS in the

following year (13.9%) compared to the 27 controls, none of whom developed IBS.[67] A prospective study of 194 conference delegates after an outbreak of norovirus showed that 23.6% of those affected had IBS like symptoms at three months, compared to 3.4% in delegates who were unaffected by the original outbreak (odds ratio 6.9). At six and twelve months from follow up the prevalence in the two groups was similar, indicating a more rapid recovery than is perhaps associated with PI-IBS after non-viral gastro-enteritis.[68] Both the patient and control groups in this prospective study of 109 subjects before and after a period of 'foreign travel' are small and the study did not find any association between traveller's diarrhoea and the development of IBS. However, as this study was small it is likely to be significantly under powered.[69]

A recent study of the aetiology of IBS in patients from three different recruitment sources, put the prevalence of PI-IBS as between 6% and 17% of total IBS cases depending on the data source used. It is also noted that this study unintentionally consisted of almost entirely female respondents and was conducted forty years after the Chauhury & Truelove publication. [70] The number of individual studies investigating PI-IBS are too numerous to mention individually. It is interesting to note however, that the majority of studies that show a clear temporal relationship between an acute gastro-enteritis and PI-IBS come from the UK, with only limited evidence in similar studies from America and Europe.[66-78] Several contradicting studies from

India and other developing countries, have failed to show any correlation between previous or current acute gastro-intestinal infections and the development of PI-IBS.[71, 72, 79] This raises the possibility that PI-IBS may be a phenomenon unique to the developed world.

Gwee et al. suggested a ‘hygiene hypothesis’ as a potential explanation for this idea.[45] They proposed that infants in a developing country are exposed to a large number of gastro-intestinal infections in early life. Accordingly it is suggested that this leads to early development of an immunological state that is both able to respond efficiently and effectively to antigenic challenges and be equally tolerant of normal, non-pathogenic gut microbes. A corresponding relative lack of, or reduced exposure to microbes in the relatively ‘sterile’ developed world; leads to a naive immune system that may be less effective at dealing with potential pathogens and at the same time have exaggerated immune mediated inflammatory responses to both pathogenic and commensal gut microbes later in life.[45] This theory is at least in part supported by the findings of increased inflammatory enteroendocrine (Ec) cells and altered levels of the pro- and anti-inflammatory mediators observed in subjects with PI-IBS [73-75, 80], which is discussed in more detail in the section on pathogenic mechanisms. The theory is also noticeable for its striking similarity with suggestions that the high prevalence of childhood asthma in developed as opposed to developing countries is dependent on microbiological or antigenic exposure.[81, 82] This

hypothesis, if not conclusive, does re-emphasise the increasing interest in the interaction of the gastrointestinal microbiota and other antigenic material with the host and the subsequent possible consequence in disease pathogenesis.

1.3.2 Food allergy and intolerance.

The acquirement of nutrients requires that food components cross the gastro-intestinal barrier and once in the systemic circulation, (in some subjects) these components may have antigenic properties that result in allergenic stimulation, immune responses and the development of allergy; which can be confirmed by challenges of the offending foodstuff and characteristic laboratory findings.[83] Intolerances on the other hand are less well understood, but much more common and consist of a clustering of symptoms that result from challenge to offending foods, but without any specific laboratory findings or evidence of a true allergenic response.

Food allergy and intolerance is frequently reported by individuals in the general population but there is poor correlation between the reported incidence and actual positive tests for food allergy. In one study with over 7000 respondents from an unselected population, 20% reported food allergy or intolerance. Of these a small number underwent specific food allergy

testing with only 19.4% having a positive result.[84] In patients with IBS, the reported incidence was even higher but a similar discrepancy and problems of interpretation is evident. In one study of patients with IBS food intolerance/allergy was reported in 32% of subjects, but of these only 14% were suspected to have allergy/intolerance based on clinical criteria; including total and specific serum IgE, provocation testing and or elimination re-challenge diets.[85] In a separate study, rates of food intolerance/allergy were as high as 63% but of these only 52% had positive 'skin-prick' tests. There was also little correlation between the positive test results and the reported food intolerance.[86] Interestingly, a systematic review that included over 4200 subjects from fourteen studies, found that those who met diagnostic criteria for IBS were four times more likely to have diagnosis of coeliac disease than the general population. [87] This is a curious finding as coeliac disease is usually the main differential diagnosis in patients with diarrhoea. A separate study also showed increased serum IgG but not IgA coeliac related antibodies (anti-gliadin and anti-tissue transglutaminase (TTG)) in 37% and HLA-DQ2 genotype in 39% of patients with D-IBS (diarrhoea predominant).[88] These findings suggest that some patients with D-IBS not only show an immunological response to dietary gluten but also that this reaction can predict their response to a gluten free diet. Furthermore, this provides direct evidence of a possible dietary trigger for low level inflammation and altered motility in a sub-group of patients with IBS and raises the possibility that similar triggers and immunological response to, as yet unidentified, dietary antigenic material may also be present.

The two most commonly reported food intolerances in patients with IBS are dairy (40.7%) intolerance; which is usually related to an intolerance to the disaccharide lactose; and wheat/grains (39.4%), commonly felt to be to the gluten component.[89] These often result in the severe bloating and diarrhoeal symptoms that are often predominant in IBS patients. Dietary fructose, and sorbitol malabsorption are also implicated in the symptomatology of IBS, as higher rates of malabsorption can be demonstrated in patients with IBS compared to controls.[90] The symptoms are presumably caused by a combination of osmotic retention of fluids resulting in loosening of stool and perhaps by providing additional substrate for fermentation by the colonic microbiome. In the hands of certain individuals symptom improvement with elimination diets fairs much better than allergy testing. In one, non-randomised, study of 189 patients with IBS, 48% showed symptomatic improvement after elimination diets for commonly reported intolerances and remained well throughout follow up. Of these 50% identified intolerances to two-five food groups. [89] In a separate study, the presence of HLA-DQ2 and increased levels of coeliac related IgG antibodies were found to predict response to gluten free diet in IBS patients.[88] Conversely in a separate study of 4622 subjects, no correlation was found between reported food intolerance, or improvement in symptoms on elimination diets with the results of food allergy testing.[91] In recent years the so called low FODMAPs diet, which stands for ‘fermentable oligo- di- and

monosaccharaides and polyols' has received considerable interest. FODMAPs elimination diets have been shown in several observational studies to improve the global symptoms of IBS. [92-94] To date there have been no true randomised-controlled trials and the methodology of the published data is unacceptable to draw any firm conclusions.

The effect of fibre intake on symptoms in IBS has been re-examined repeatedly with a variety of changing messages. There is no significant data in the published literature examining the role of variations in dietary fibre intake in the aetiology of IBS. The use of dietary fibre as a treatment strategy is discussed later.

The evidence for dietary intolerances and food allergy in the context of the aetiology and pathogenesis of IBS is inconclusive with conflicting results from different studies. Clearly, as is often the case, there are proponents and opponents to dietary interventions as a therapeutic target for IBS and this may have some merit. However, the evidence that specific food intolerance and allergy play a role in the pathogenesis of IBS is unconvincing. This does not necessarily preclude the use of elimination diets in the treatment of IBS, where perhaps there is at least some evidence, but even then there is a lack of randomised controlled trial data. The complex physiological changes that occur as a response to the stimulation triggered by the presence of food

within the GI tract are a normal phenomenon. There is also a multitude of evidence to suggest disordered physiological response and visceral hypersensitivity in patients with IBS. If these physiological changes are exaggerated or result in symptoms in IBS it is not, in my opinion and based on the evidence available, appropriate to define this in the vast majority of patients with IBS as food allergy or intolerance. Dietary manipulation and elimination diets remain an important part of current treatments for IBS but perhaps as our understanding of the pathophysiology and pathogenesis of

the disease increase, so too will our understanding of the impact of dietary components.

1.3.3 Psychosocial factors in the aetiology of irritable bowel syndrome

Psychological factors are thought to play both an important role in the development of IBS and possibly may even play a direct contributory role in the disease pathogenesis. Certainly it is clearly established that patients with IBS who consult their doctor have an increased incidence of psychological co-morbidities and traits than either the general population, or patients with IBS who do not seek to consult their healthcare practitioner.[57]

The aetiology and natural history of IBS as well as the response to treatment is significantly influenced by psychological factors in most if not all patients. However, this interaction may have been misrepresented or over interpreted in the literature and may contribute to the misunderstanding of the disease in the clinical setting resulting in poor treatment outcomes. Chaudhury and Truelove identified psychological factors in 80% of 130 patients and subdivided these factors into; diagnosable psychiatric illness, anxious personality type and those suffering from environmental stress such as family, marital or work related.[3] This high prevalence of psychiatric conditions led the authors and others to conclude that psychological factors were a key component in the aetiology of IBS. Creed and Guthrie 1987 on the other hand highlight that there was a high likelihood that poor methodology in this and other earlier studies might lead to erroneous interpretation. These include significant selection biases of patients, failure to use standardised diagnostic classifications and failure to use, or inappropriate use of, reliable psychological instruments which predisposes to significant overestimation of the incidence of psychological abnormalities associated with IBS.[55] Furthermore, and much more importantly, the study does not address whether there is a temporal or causal relationship between IBS and the psychiatric parameters. The high prevalence reported in what is often referred to as a ‘landmark’ study is still used to support the theory of a psychosocial disease pathogenesis in IBS despite its clear failings.

Some studies have shown a higher incidence of divorce/separation, alcoholism, childhood deprivation, unsatisfactory relationships in childhood with / between parents [95] and a higher prevalence of sexual abuse in subjects with IBS compared to non-neoplastic organic disease.[96, 97] Hislop et al. in an observational study of 333 consecutive patients with IBS noted 31% had either lost a parent through death, divorce or separation, 19% were exposed to parental alcoholism and 61% felt that relationships with or between parents were unsatisfactory before the age of 15.[95] This study did not include a control group. A multi-centre, prospective, case control study of 196 patients (200 controls) with IBS who attended outpatients in a teaching hospital found that patients with IBS reported a significantly higher rate of sexual abuse (31.6%), than controls (14.0%).[96] However, the patient group was selected from teaching hospital outpatient clinics only. As psychological abnormalities are more common in IBS patients who seek medical consultation,[56] it is possible the population studied are not representative of patients with IBS as a whole. These studies are invariably retrospective as they ask the individuals to recall past experience and do not therefore adequately demonstrate either a causal or direct temporal relationship.

Patients who develop IBS after an acute GI infection have not been thought to have a particular premorbid personality. However, Spence et al. demonstrated in their prospective study of 620 patients with campylobacter

gastroenteritis, that those patients who go on to develop post infectious IBS (PI-IBS) have significantly higher levels of stress and anxiety at the time of the primary infection than patients who do not.[98] A second similar prospective study of 75 patients by Gwee et al. also shows higher scores for anxiety, depression, somatisation and neurotic traits on psychometric testing, at the time of the infective illness in those patients who go on to develop PI-IBS.[99] The authors of both studies suggest that this reflects a causative role of the identified characteristics. However, whilst these studies are interesting and suggest a causative and temporal relationship between psychological factors and the development of IBS, they utilise questionnaires to report the psychiatric traits and the IBS like symptoms. Due to the semi-subjective nature of these questionnaires, self-reporting and response bias (25% non-response in one study), these results should be viewed with some caution. Drossman et al. 1998 studied a cohort of 238 subjects, 72 patients with IBS who regularly consulted their physician, 82 IBS 'non-patients' (patients who met the diagnostic criteria for IBS but did not regularly consult a physician) and 84 healthy controls without IBS. They demonstrated a higher incidence of psychological factors including abnormal personality traits, disruptive illness behaviour and lower positive stressful life event scores in the IBS patient group than either the 'non patients' or controls. IBS 'non-patients' were intermediate between the two groups but tended to have a higher coping ability, and less disruptive illness behaviour or psychological denial [57]. This study suggests that the abnormal psychological traits are a feature of the presentation to healthcare rather than the underlying disease

itself. Similarly, Akehurst et al. 2002 showed increased healthcare utilisation and costs in a cohort of patients with IBS compared with 213 age matched healthy controls. This also corresponded with lower health related quality of life scores (HR-QOL) reported in the IBS patient group.[65] Hershbach et al. 1999 further supported this view with similar findings, that people with IBS do not differ from controls but patients with IBS have more psychopathology, fear of illness and are more markedly affected by stress concluding that the psychopathology is a not a characteristic of the disorder but a sampling bias.[60] Conflictingly, Heaton 1992 demonstrated that the number and severity of IBS symptoms predicted health seeking behaviour independently of psychological factors.[7]

It is apparent from the literature that the interaction between the psychological factors (which are undoubtedly associated with IBS) and the physical symptoms in patients with IBS is complex. It appears that they are both important in the pathophysiology and health seeking behaviour of IBS patients. Whether they are truly part of the disease aetiology or simply a trait that leads to consultation continues to divide opinion. Some patients with quite severe IBS have no apparent underlying psychological problems and the disparity between the frequency of psychological problems in patients seen in secondary and tertiary care settings compared to those in the community or primary care setting would suggest that it is a feature of presentation and not the disease itself. However, the temporal relationship

observed between psychological symptoms and development of PI-IBS suggest at least some role in pathogenesis.

1.4 Pathogenesis and underlying mechanisms.

The causes for IBS are unknown and speculative. The genetic and psychosocial influences have been discussed previously. Historically IBS has been regarded as a condition in which altered gut motility, visceral hypersensitivity and psychological factors are the key pathogenic mechanisms. More recent research now suggests that autonomic, enteric and central nervous system dysfunction, altered immune function and disordered inflammatory responses are also key pathogenic mechanisms in IBS. These pathogenic mechanisms are the result of a combination of various genetic and environmental influences with each, if not all, of the mechanistic components interacting and contributing to a varying degree, depending on the aetiology and sub-type of IBS encountered.

An understanding of the complex physiology and function of the organs that are collectively referred to as the gastro-intestinal (GI) tract is essential when considering the pathogenic mechanisms of IBS. The GI tract does not merely function as an organ to facilitate transfer of nutrients from the lumen to the systemic circulation, but also acts as a complex barrier with

non-specific defence mechanisms as well innate and adaptive immunological components that protect against invasion of microbes and other luminal antigens.

1.4.1 Brain-Gut Axis: Visceral hypersensitivity, hyperalgesia and central pain perception.

The gastrointestinal tract is innervated both by intrinsic and extrinsic nervous systems. The brain-gut axis commences with signals arising from the intrinsic enteric nervous system within the gut wall being transmitted to via various visceral afferent pathways, including the enteric, spinal and vagal pathways to the central nervous system.[100] The brain-gut axis within the CNS involves complex communications between a variety of structures, including the limbic sensory and motor cortex, hypothalamus, mesencephalic and medullary systems.[101] This complex system allows for physiological and homeostatic reflex to occur directly within the ENS, as well as through more complex interactions and reflexes originating in and/or under the influence of the central nervous system.[102] It is through these mechanisms that regulation of basic GI physiology such as blood flow and secretion are regulated, as well as the much more complex integration and co-ordination of gut physiology within the wider homeostatic requirements and mechanisms of the body.[101] In addition, the complex interactions with the CNS also play

a role in the satiety, pain and emotional and immune responses, [103] as well as adjusting the sensitivity of these reflexes and visceral pain sensation.[104] Whilst the vast majority of the reflex interactions and control of gut physiology and function are not consciously either perceived or controlled in normal subjects, there are central and peripheral adaptive mechanisms that alter the perception of visceral stimuli.[101] These include inflammation, tissue injury, food and luminal antigenic material as well as emotional stressors; all of which can directly and indirectly influence gut-physiology and perception of stimuli via the gut-brain axis.[100, 105-108]

The term visceral hypersensitivity describes a state in which a subject reports a painful or uncomfortable sensation to a physiological or other stimulus that would not usually be considered painful by a healthy subject. It was first described by Richie et al. who demonstrated a hyperalgesic response to balloon distension within the pelvic colon in subjects with IBS compared to controls.[109] Over the last decade or so the underlying mechanisms of visceral hypersensitivity are becoming increasingly understood; as a result it is becoming clear that it is one of the key mechanisms that underpins the abdominal pain and discomfort that is reported in IBS patients. The exact underlying mechanisms and the degree of involvement of the brain-gut axis in the development of IBS is still being unearthed. Structural and functional abnormalities of anterior mid-singulate and insular cortex have been suggested by two MRI studies.[110, 111]

Further differences are proposed in terms of regional changes in grey matter density [111] but as yet it is not known whether these changes are predisposing to; part of the pathophysiology of, or a result of repetitive visceral stimulation in the context of IBS.[112] Further disturbance in the interaction between the CNS and the autonomic nervous system (ANS) mediated in principle via corticotrophin releasing factor (CRF) and the CRF-1 and CRF-2 receptors, may also result in increased perception of both gastrointestinal and non-gastrointestinal symptoms in IBS patients. [113, 114]

The exact pathogenesis and mechanisms of visceral hypersensitivity itself are not known, although there are several theories such as increased sensitisation as a result of repeated mucosal injury and/or inflammation.[105] The visceral afferent fibres of the gut lumen are polymodal and triggered by local luminal chemical, thermal and mechanical stimuli.[115] The sensitivity of so called ‘silent’ mechanoreceptors can be increased by mucosal injury such as the instillation of deoxycolic acid in a rat model and may persist long after the removal of the initial insult and resultant inflammation.[116] Injury to the enteric mucosa leads to the production and release of a host of chemical (potassium, adenosine triphosphate (ATP) and bradykinin) and inflammatory (prostaglandin E_2 (PGE_2)) mediators. [117] In addition, to direct afferent nerve stimulation these substances can also induce the release of histamine, serotonin, nerve growth factor and prostaglandins leading to

further amplification of the stimulus resultant in visceral pain.[118] Serotonin appears to play a particularly important role in stimulating primary afferent nerve endings and perpetuating the visceral pain response. [119]. In addition to local mediators of pain, significant central sensitisation to visceral pain response is also implicated. This takes place both at the level of the spinal cord mediated via substance P, neurokinin 1, and N-methyl-D-aspartate (NMDA) receptors; and centrally via gamma-aminobutyric acid GABA and serotonergic pathways.[120] Information from animal models is vital to the understanding of the pathophysiological mechanisms underlying the development of visceral hyperalgesic states. Abnormal somatic pain in IBS is equally essential to elucidate, but hampered by technical difficulties. Nevertheless information from clinical studies is progressing our knowledge of pain albeit slowly. One way forward in these respects is the use of objective assessment such as functional MRI in the investigation of the consequence of a physiological and painful stimuli, such as thermal skin exposure and rectal distension.

A study of twenty patients with IBS and fourteen healthy controls looked at responses to rectal balloon distension on five occasions over a period of twelve months. In the IBS group positron emission tomography mapping showed increased limbic, paralimbic and pontine stimulation consistent with central up regulation/arousal of response that corresponded with increased perceptual rating scores, suggesting a degree of habituation

to visceral stimuli that was not present in normal controls.[121] Abnormal pain sensitisation to somatic stimulation is also a feature of IBS, although whether this is due to central pain sensitisation of viscera-somatic convergence sensitisation as a result of the visceral insult is not clear.[122] A possible mechanism for the somatic sensitisation could be the same as that seen in chronic neuropathic pain; where persistent afferent nerve stimulation of the spinal cord leads to hyperalgesia, pain and allodynia.[105] A further clinical study demonstrated the phenomenon of temporal summation of pain in patients with IBS compared to controls, where repeat exposure to the same noxious, thermal stimulus resulted in exaggerated somatic pain response that could be blocked by dextromethorphan, an NMDA receptor antagonist.[123]

Clearly, our understanding of the central and peripheral components that lead to visceral hypersensitivity and hyperalgesia is relatively poor, but the combination of central arousal and habitualisation, together with sensitisation of peripheral afferent nerves may, at least in part, be responsible.

1.4.2 Genetic Factors.

The possibility of one or more genetic factors that directly or indirectly, through increased susceptibility, are involved in the pathogenesis of IBS is raised by the observation of familial aggregation of IBS.[124, 125] In a prospective study of 355 patients with IBS, 17% had one or more family members with IBS like symptoms compared to 7% in the family of the spouse.[124] However, familial aggregation alone is insufficient evidence to suggest a genetic component, as it may simply be representative of a common exposure to environmental factors.

Several twin studies have been conducted in patients with IBS, but the results are conflicting both with each other and the familial aggregation studies. Two twin studies conducted by Bengston et al. and Levy et al. of 12,700 and 10,609 twins, respectively, reported greater concordance of IBS in monozygotic (22.4% and 17.2%) than dizygotic twins (9.1% and 8.4%) respectively.[126, 127] Bengston et al. also reported 48.4% concordance in female monozygotic twins which is very suggestive of a genetic component. But then goes to report a higher incidence of IBS in low birth weight twins suggesting an intra-uterine environmental factor may also be of significant importance.[126] Levy et al. reported a higher proportion of dizygotic twins with a mother (15.2%) with IBS than a co-twin (6.7%) This raises further questions, as the amount of genetic information shared between mother and

offspring and co-twin (dizygotic) is equal and as such the proportions should be similar.[127] A further study from Mohammed et al. of 5,032 twins reported equal concordance, but with a much higher concordance than either Bengsten et al. or Levy et al., in monozygotic and dizygotic twins (28% and 27%) with similar prevalence suggesting that a genetic factor is unlikely [128]. All three studies rely on self-reporting of IBS by answering yes/no to a simple question and do not employ any recognised diagnostic criteria to confirm the diagnosis, which limits the relevance of the results. These genetic studies perhaps raise more questions than they answer.

The importance of adrenoceptors and serotonin in the pathogenesis of IBS is discussed later, but it is essential to note that over 95% of serotonin is located in the enteric nervous system. Within the enteric nervous system there are two distinct seritonergic pathways; the intrinsic and extrinsic pathway, which are mediated principally through 5-HT_4 and 5-HT_3 receptors respectively. The intrinsic pathway relates mainly to peristaltic reflexes, motility and secretory functions, whereas the extrinsic pathway relates to visceral sensation and nociceptive pathways[129]. Genetic polymorphisms in $alpha_2$-adrenoceptor and the serotonin transporter (SERT) have been linked to different subtypes of IBS and as such perhaps give the strongest evidence of a link to a genetic component yet.[130, 131]

A study of 230 patients with IBS and 430 healthy controls, found that there was a significantly lower frequency of the high producer interleukin 10 (IL-10) and tumour necrosis factor alpha (TNF_{α}) genotypes (21% versus 32% (P = 0.003)) in patients with IBS.[74] A further study confirms this result, reporting significantly more patients with IBS (9%) than controls (3%)(P = 0.035) with a high TNF_{α}, low IL-10 genotype. Genetic polymorphisms in TNF_{α} and IL-10 polymorphisms linked with IBS provide further evidence and may contribute to some of the disordered or exaggerated inflammatory to luminal antigens seen in IBS.[74, 132]

An ever increasing body of evidence seems to support the presence of several genetic factors implicated directly in the pathogenesis and/or susceptibility to the development of IBS. However, the degree of influence of these genetics components, although not clear, is likely to be modest and further large population based studies are required.

1.4.3 Infection, Inflammation and mucosal injury.

The role of infection in the cause of PI-IBS has already been discussed and whilst there is some inconsistency in results from different populations and with different types of infection, it would appear that there is, at least some of the time, an infective trigger to the development of IBS (PI-

IBS) in a sub-group of patients. What is less clear is the role of enteric infections and commensal bacteria in the underlying pathophysiology of all IBS cases. The development and maintenance of a functioning and immune-competent gut mucosa is dependent on the complex interactions between the luminal microbiome and the host, but this is discussed in more detail later.

The host mucosal and immune-response to an enteric infection may result in changes that persist long after the original infection in IBS patients. In a prospective study by Spiller et al. serial rectal biopsies following infection with Campylobacter enteritis were performed in patients with PI-IBS and a healthy control group. The PI-IBS group had significantly increased small intestinal permeability, higher numbers of enteroendocrine cells, intraepithelial lymphocytes and CD3, CD4 and CD8 within the lamina propria of the gastrointestinal mucosa than the healthy controls in the first three months after the infection and some changes persisted one year later.[80] A further study by Dunlop et al. also reported higher numbers of enterochromaffin cells and T-lymphocytes in patients three months after Campylobacter enteritis in patients who developed PI-IBS compared to those who did not have persisting symptoms.[73] Gwee et al. also noted significantly greater expression of the pro-inflammatory cytokine IL-1β three months after acute gastroenteritis in patients with PI-IBS than in normal controls.[75] The increased mucosal permeability also noted by Spiller et al

in PI-IBS patients may also serve to drive the inflammatory response by exposing the intra- and sub-mucosal elements to luminal antigenic material.[80] Goral et al. identified higher serum levels of the pro-inflammatory cytokine Il-2 and an increased number of mast cells in colonic biopsies in D-IBS compared to C-IBD and normal controls.[133] Han et al. have also shown that soluble factors taken from colonic biopsies of PI-IBS patients can activate peritoneal mast cells in vitro and significantly increase protein-activated receptor (PAR(2)) mRNA (a mediator of hyperalgesia) expression.[134] Genetic factors that influence the immune-inflammatory response to enteric infections are also likely to be important. Villani et al describe four specific genetic variants associated with PI- IBS, two located in TLR9 encoding for a 'pattern recognition' receptor; one at CDH1 encoding a tight junction protein and one in IL6 encoding a cytokine. IL-10 and TNFα polyporhisms are also both associated with IBS.[132]

Pro-inflammatory genetic variants and persisting inflammatory mucosal changes in response to acute enteric infections in patients with IBS, certainly point towards an infective or inflammatory mediated disease in PI-IBS. However, whether the mucosal injury and persisting inflammation is the result of a pre-existing abnormal pro-inflammatory state of the host or an infection mediated phenomenon is not clear. It has also been shown in animal models that inflicted stress by either early maternal separation or limited restraint can also induce mucosal barrier dysfunction and increased

permeability.[135, 136] Furthermore, it is probably a reasonable assumption to state that all adults at some time in the past will have experienced at least one, and likely more, enteric infections.

Therefore, in my opinion, even when a direct temporal relationship between the infective insult on the gut mucosa and the development of IBS symptoms is not evident, it would be wrong to conclude the IBS as non-infective. It is entirely possible that accumulative previous infective enteric and perhaps even non-enteric events may be contributory in the pathogenesis of all IBS symptoms. Again the lack of animal models for IBS and scarcity of data in man hampers interpretation.

1.5 Clinical Practice: Classification and Diagnosis

The importance of the initial consultation in the diagnosis cannot be overstated, as the lack of a specific diagnostic test(s) for IBS means that the skill of the physician in acquiring a comprehensive medical and psychosocial history together with a full physical examination is vital. Allowing sufficient time during the initial consultation to establishment a good rapport, obtain the history and explore the patient thoughts and ideas is equally as important and is perhaps the key to a successful working ‘doctor-patient’ relationship. It is often the case that patient’s have suffered from symptoms for a

considerable amount of time before seeking advice, or may even have had several unrewarding consultations or unsuccessful treatments in the past. Patients are further frustrated by the fact that many gastroenterologists and some general physicians are content to reach the diagnosis without providing much in the way of treatment as this is so often a laborious process.

1.5.1 Clinical definition and classification

In clinical practice the term ‘IBS’ if often loosely applied to any patient that has a functional gastro-intestinal disorder (FGID). Inappropriate and inaccurate application of the diagnosis ‘IBS’ may result in confusion for clinicians and patients alike and result in insufficient or ineffective treatment. The Rome Foundation was created in order to address this and other issues around the treatment/management and classification of FGIDs. The most recent Rome criteria (ROME III) were published in 2006.[4] For research purposes it is essential to use defined diagnostic criteria to ensure the validity and comparability of research findings. The most commonly used are the Rome II or Rome III criteria (Table 1). Further classification of IBS based on predominant stool consistency according to the Bristol Stool Form Scale [137] results in four subtypes: C-IBS (constipation), D-IBS (diarrhoea), M-IBS (mixed) IBS and U (unsubtyped) [4] (Table 2)

1.5.2 Diagnosis

The belief that IBS is a diagnosis of exclusion should be confined to the history books and yet, despite recent guidelines repeatedly emphasising the need for a positive clinical diagnosis, [138] up to 72% of non-expert clinicians compared to 8% of experts uphold the idea of a diagnosis of exclusion. [139] A clinician with an interest in IBS, who is knowledgeable about functional gastro-intestinal disorders, can be reasonably confident of making a diagnosis of IBS based on history and examination alone. However, in clinical practice it is usual to include some baseline non-invasive laboratory investigations to support the clinician's diagnosis of IBS and exclude other disorders. These investigations should be limited to simple non-invasive laboratory based investigations which are sensitive for excluding organic intestinal disease. Invasive investigations including colonoscopy should be recommended only in the presence of 'red flag' symptoms, abnormal laboratory tests or if the clinician strongly suspects an alternative diagnosis.

Occasionally, it can be extremely difficult to reassure a patient about the absence of organic disease without undertaking a colonoscopy. In such cases, even though performing a colonoscopy may seem the easiest and

most appropriate option, it should not be undertaken lightly and may increase patient anxieties about diagnostic uncertainty. Colonoscopy is not without well recognised clinical risks and particularly in patients with IBS, as a result of the inherent visceral hypersensitivity, it can often be a very painful and unpleasant procedure. Furthermore, if an IBS patient experiences a painful colonoscopy and has psychological co-morbidities, it may ultimately result in worsening psychological and physical symptoms as well as a worsening belief that something 'more serious' has been missed due to the pain experienced. If a colonoscopy is undertaken, it is essential that the patients' expectations are managed appropriately and they are informed prior to the procedure that a normal result is expected.

The presenting history of IBS will vary considerably between cases, but to satisfy diagnostic criteria the symptoms need to have started at least two months previously and include abdominal pain/discomfort (a sensation that is not comfortable but falls short of pain) occurring on at least one day of each week (Rome III criteria: table 1). The abdominal discomfort/pain is associated with a change in the frequency and consistency of bowel movements and is relieved by defecation at least some of the time. The disordered bowel habit may change over time but commonly includes either or both diarrhoea and constipation in varying proportions. The symptoms of urgency of bowel movement (common in patients with ulcerative colitis); feeling of incomplete evacuation (also seen in patients with rectocele);

bloating; prominent gastro-colic reflex (sensation to defecate shortly after meals); increased flatulence and passage of mucus per rectum are all associated with IBS, but are not essential features nor are they sufficient alone to make a diagnosis. Extra-intestinal manifestations are also a frequent but non-essential feature in many patients and include; lethargy, poor concentration, poor appetite, anxiety, joint and muscular pain, headaches' urinary symptoms, menstrual irregularity and difficulty sleeping.

The 'red flag' symptoms of passing blood per rectum, iron deficiency anaemia, weight loss and nocturnal bowel movements all raise the possibility of an alternative diagnosis including inflammatory bowel disease and gasto-intestinal malignancy.

1.6 Treatment options for IBS in clinical practice.

The currently available treatment for IBS is centred on symptom control. There is no conclusive evidence to suggest that if left untreated the symptoms of IBS will deteriorate and IBS alone is never directly fatal. However, the long term psychological effects of untreated IBS are not known. Furthermore, the long-term consequences of disordered gut physiology as well as repeated painful stimuli themselves, although not proven, may have a harmful effect. As such care should be given when

choosing a treatment for IBS with safety and side effects of any treatment arguably remaining the highest priority.[138] Many different treatments are available for IBS but the efficacy is limited and in some cases there is lack of proof to the efficacy by appropriately designed trials. In general, treatment strategies include conventional medicines, dietary manipulation, psychological therapy and alternative treatments.

1.6.1 Dietary Treatments.

1.6.1.1 Dietary Fibre Intake

An increase in dietary fibre intake has frequently been advocated to improve the symptoms of IBS, (especially in constipation predominant IBS) but the evidence to support this is limited. Fibre exists in two principle forms; soluble fibre (including psyllium (ispaghula) and pectins) and insoluble (bran and cereal husks). Insoluble fibre, is a chemically inert substance and passes through the GI tract largely unchanged, It retains water within the gut lumen by osmosis and in theory acts as a stool bulking agent. Soluble fibre dissolves in the luminal contents and provides a fermentation substrate that releases short chain fatty acids and gas to be used and as such may increase gut motility and improve constipation.

The use of all types of dietary fibre is controversial but still advocated by many clinicians in the treatment of IBS. However, the evidence for this is limited and some studies suggest that it may even worsen symptoms. Francis et al., in a study of 100 consecutive IBS patients from their outpatient unit, found that 55% reported a worsening of all symptoms but especially bowel disturbance followed by distension and pain associated with bran supplements and only 10% found any improvement.[140] A systematic review of the use of psyllium in IBS patients identified twelve studies met their inclusion criteria, but that the methodology and study designs were heterogeneous. The results of the study were conflicting with only half showing improvement in global symptoms scores and one showing an improvement in pain. Quality of life and flatulence scores did not improve.[141] A second systematic-review including seventeen studies of both soluble an insoluble fibre found that fibre was efficacious in improving global symptoms in 60% of patients but not abdominal pain, which may be worsened in some patients and that overall there was no significant difference from placebo. [142] The authors of both studies concluded that there was insufficient evidence to support the recommendation of fibre as a treatment for IBS.[141, 142]

1.6.1.2 Elimination diets

Food intolerance and food allergies are frequently self-reported with up to 20% of the general population [84] and 32-63% of IBS patients,[85, 86] reporting one or more food intolerances. However, in these studies there was significant disparity in self-reporting intolerances, the results of IgG allergy testing and response to elimination diets.[84-86] The principle of IgG testing in IBS patients is to establish the presence of the 'true' immune mediated allergic response to antigenic food components rather than a physiological intolerance as in lactose intolerance. In doing so it is hoped that IgG testing results will provide a basis for food elimination diets. The use of elimination diets in the treatment of IBS is common in clinical practice; whether this is evidence based practice is not clear. Drisko et al. studied twenty patients who had not responded to conventional treatments for IBS with elimination diets based on the results of IgG food allergy testing and found significant improvement in abdominal pain, stool frequency and IBS-quality of life (QOL) scores.[143] A second study by Zat et al, of 25 patients with IBS, also found improvements in pain, bloating, bowel habit satisfaction and overall QOL after treatment, with elimination diets based on IgG food allergy testing.[144] However, both studies were small, non-randomised and did not include a placebo arm.

Lactose and fructose intolerance are one of the more common reported in IBS. Corley et al. in a study of 181 subjects found an equal

prevalence of fructose/lactose intolerance of 33% in both IBS patients and healthy controls, based on hydrogen/methane breath testing in response to an oral carbohydrate challenge. They also found that whilst the IBS patients were more likely to report symptoms associated with the food intolerance, only 47% had improvement in symptom compared to 77% of controls.[145] A second study by Goldstein et al. looked at combined lactose, fructose and sorbitol intolerance in 94 patients with IBS and 145 patients with IBS like symptoms, that did not fulfil the Rome criteria and found that 56% and 60% of patients reported symptom improvement on elimination of the offending sugar(s). [146] Again, neither study was randomised nor placebo controlled, in addition the method for diagnosing lactose/fructose intolerance is frequently criticised.

Wheat intolerance is also frequently encountered in IBS patients with one large systematic-review finding that patients who met diagnostic criteria for IBS were four times more likely to have biopsy proven coeliac disease.[87] Such patients are effectively irrelevant in the context of treatment of IBS and have been wrongly diagnosed. However, those that remain (and who do not have coeliac disease) often appear to show some improvement on a wheat free diet. Wanschaffe et al. found that in D-IBS patients who were positive for coeliac IgG but not IgA antibody and thus not consistent with true coeliac disease; and had HLA-DQ2 expression, had significantly better symptoms improvement (60%) than those who were

negative (12%) on wheat exclusion diets.[88] Unsurprisingly, this study was not placebo controlled but does appear to demonstrate that response to wheat free diet can be predicted on the basis of serum parameters.

A randomised controlled trial in which 160 patients with IBS received either a ‘sham-diet’ or an elimination diet, based on the positive IgG responses to food allergy testing also reported a positive outcome. This study found that symptom improvement, rated by a global rating scale was 10% higher in the ‘true’ diet compared to the ‘sham’ diet.[147] Whilst, this study did attempt to have a control/placebo arm by introducing the ‘sham’ diet, it was heavily criticised as the diets were markedly different; and the true diet, when compared to the ‘sham’ diet excluded substantially more foodstuffs that are commonly reported as food intolerances in IBS patients. The level of reported IgG positive food allergy was also exceptionally high when compared to other studies.

Perhaps the most extreme elimination diet is the so-called low FODMAP diet (fermentable oligo- di- and monosaccharides and polyols) in which patients eliminate a large variety of different foods. The evidence for this diet is limited to a few studies, including the original study of 62 consecutive non-randomised patients in a double-blind, placebo controlled, re-challenge design. This study by Shephard et al. found that 77% and 79%

of patients who received fructose and fructans respectively reported that the symptoms were not adequately controlled.[148] The methodology of this study is extremely limited both by its size, lack of randomisation and crossover design. In addition, there is no discussion of whether a validated instrument is used to assess outcome measures and the result compares diet adherent and non-adherent patient outcomes. A recent study of 82 patients in a UK institution comparing low FODMAP with standard dietetic therapy diet reported a significant improvement in composite symptoms scores of 86% vs. 49% respectively (P = <0.001) and satisfaction with symptom improvement 76% vs. 54% (P = 0.038).[92] This study compares retrospective data on standard dietary therapy with data from patients prospectively treated with low FODMAP diet and whilst it demonstrates superiority over standard therapy the study design has significant methodological limitations. The study was not blinded or randomised; contained no placebo or sham arm, combined retrospective and prospective data, the outcome measure was not validated and the research group failed to classify IBS patients according to any recognised classification. The results must therefore be viewed with marked caution.

Dieticians frequently play a major role in the treatment of patients with IBS and are usually a member the clinical multi-disciplinary team for patients with IBS. Whilst their role in IBS treatment is clinically important, the evidence for the vast majority of dietary interventions is lacking. The

inherent methodological limitations of dietary interventional studies make it difficult to come to any firm conclusions about diet as a treatment for IBS. However, the same can also be said about many other commonly used interventions in IBS, many of which lack conclusive evidence of efficacy.

1.6.2 Conventional medicines for the treatment of IBS.

There are a multitude of available conventional medications available as both ‘over the counter’ remedies and those that require a medical prescription. The evidence of efficacy for individual medications is often variable and for some agents the side effect profile may limit the use in a substantial number of patients. The following section reviews the evidence for medical therapies commonly used but is not intended to be an exhaustive review of the available data.

1.621 Antispasmodics.

Antispasmodic medications are commonly used in both primary and secondary care for the treatment of IBS and as such have remained one of the key prescribed medications for some time. Those available in the United Kingdom (UK) include alverine, dicyloverine, mebeverine, peppermint oil and hyoscine butylbromide (a scopolamine derivative). Other antispasmodics not available or not licensed for the treatment of IBS in the UK include pinavrium, pirenzepine, propinox and trimebutine.

Mebeverine, arguably the most commonly prescribed antispasmodic in the UK has not been shown to be efficacious in the improvement of global symptom severity in patients with IBS. Kruis et al. in an RCT of 80 patients did not show any difference in improvement in global symptom severity score of mebeverine over placebo, (RR 0.83; 85% CI: 0.31 to 2.23). [149] Moreover, a recent meta-analysis with a total of 555 patients did not find any benefit of mebeverine over placebo in controlling global symptom severity (RR 1.13 95% CI: 0.59-2.16, P = 0.71).[150] A further study of 712 IBS patients using hyoscine butylbromide and hyoscine butylbromide in combination with paracetamol demonstrated superiority in both arms over placebo.[151] A systematic review of the use of smooth muscle relaxants in IBS which included 23 studies and 1888 patients in total, concluded that they were superior to placebo in the treatment of IBS (OR 1.65; 95% CI: 1.30-2.10, P < 0.001). [152] This systematic review included data from studies

using several different smooth muscle relaxants/antispasmodics and therefore the results may not be applicable to the individual drugs. Lastly, it is important to consider that side effects, whilst usually not severe, are not uncommon in patients using these medications which in the context of unproven efficacy may limit their usefulness.

1.6.2.2 Anti-depressants.

The role and mechanisms of action of anti-depressants and drugs that act via serotonin pathways in the treatment of IBS is complex. Anti-depressants are frequently used in clinical practice and have been shown to be useful in the management of IBS and other chronic pain conditions via their peripheral neuromodulatory and analgesic as well as central effects on pain modulation and perception.[153-156] There is evidence from clinical trials for the use of individual agents including imipramine (desipramine) amitriptyline, paroxetine, fluoxetine and citalopram for the treatment of IBS.

Rajagopalan et al. in 1998 studied 40 patients with IBS, defined by the Rome criteria, in a randomised controlled trial of placebo versus amytriptylline (at incremental doses of 25mg for one week, 50mg for one week and 75mg thereafter for twelve weeks). 22 patients completed the study and the authors report a significant improvement in global symptom

scores at twelve weeks in the treatment group (63.6%) compared to placebo (25.9%).[157] Greenbaum et al. studied 41 patients with a clinical but unclassified (no diagnostic criteria given) IBS in a double blind crossover study with desipramine, atropine and placebo. 28 patients completed three, six week periods of treatment and reported a significant improvement in the global symptom severity score during treatment with desipramine.[158] Both studies have significant methodological limitations and do not use standardised outcomes measures, although these were unlikely to be available at the time the studies were conducted. Both studies suffer from high dropout rates with little explanation as to the reasons and subsequent exclusion of these patients from the efficacy analysis, as was standard practice at the time. These methodological limitations and the sizes of the studies significantly limit the application and validity of the results. A later randomised, double-blind study of imipramine versus placebo by Abdul-Baki et al. included 107 Rome II, IBS patients but significant drop outs during the sixteen week study resulted in only 56 (31 treatment, 25 placebo) completing the study. The study was closed early and only a retrospective ad-hoc power calculation was performed. Patients on imipramine showed a greater improvement in both QOL and symptoms severity scores but failed to reach significance, perhaps due to the very high dropout rate in both the placebo and treatment group and the small study cohort. [159] Vahedi et al. undertook a study of amitriptyline in 54 patients with D-IBS showing a significant improvement in all symptoms in the treatment group (68%)

versus placebo (28%)(p = 0.001) but did not use a validated outcome measure and simply relied on binary outcomes to symptom reporting.[160]

A double blind placebo controlled crossover trial of 23 patients demonstrated superiority of citalopram over placebo in global symptom score, abdominal pain, and severity of impact of symptoms on daily life, (P < 0.05)[161] but has been criticised for its size of cohort and crossover design . A further study of citalopram versus imipramine and placebo in 51 patients failed to show superiority of either citalopram or imipramine over placebo in improvement in global symptom severity score, but did show an improvement in severity of interference of symptoms(P = 0.05), distress (P = 0.02) for imipramine. [162]

Several meta-analysis of tricyclic antidepressants have also been conducted. One identifies seven eligible randomised control trials, including some of the aforementioned studies, of reasonable quality. It reports a pooled relative risk (RR) of 1.93 for an improvement in symptoms with tri-cyclic antidepressants compared to placebo (p <0.0001)[163]. A further systemic review and meta-analysis including nine RCTs for tri-cyclic anti-depressants and five for serotonin re-uptake inhibitors (SSRIs) found an RR of 0.68 (95% CI: 0.56 – 0.83) (P = 0.001) and 0.62 (CI: 0.45 – 0.87)(P =

0.006) respectively and an overall effect RR of 0.66 (CI: 0.57 – 0.78)($p < 0.00001$) favouring treatment with anti-depressants. [164]

Whilst the results of some of the trials for anti-depressant usage in IBS are conflicting and often the size of said studies methodologies are limiting; the meta-analyses are favourable and in clinical practice they are often used to reasonably good effect. However, the side effects of drowsiness, sleep disturbance, daytime somnolence, increased anxiety and reduced concentration are not infrequent. Whilst these side effects often reduce after a short time period, in practice they often limit the tolerability and acceptability of the treatment.

1.6.2.3 Serotonin receptor agonists and antagonists.

Serotonin has a key role in the physiological mechanisms over and above those related to pain and visceral hypersensitivity. As a result the $5HT_3$ and $5HT_4$ have been utilised as therapeutic targets for the treatment of IBS. The $5HT_4$ agonist tergaserod was shown to be superior to placebo in several clinical trials at improving abdominal pain and global symptom severity in patients with C-IBS, but not quality of life.[165, 166] A subsequent systematic review of 11RCTS including 9242 subjects also concluded that it was superior to placebo (RR 0.85, CI: 0.80 to 0.90). [167] However, in 2007

it was withdrawn because of concerns over increased risk of ischaemic cerebrovascular, cardiovascular events and severe diarrhoea.

The $5HT_3$ agonist, Alosetron has been evaluated in several studies and shown to be efficacious in treating some symptoms of IBS. A systematic review of the data, including eight RCTS and a total of 4987 patients comparing Alosetron with placebo concluded that it may be effective in controlling abdominal pain and improving global symptom severity (RR 0.79 95% CI: 0.69 – 0.90; $P < 0.00001$). [167] However, it is also of note that two of the included studies only evaluated D-IBS and four included cohorts with at least 70% D-IBS. Furthermore, five excluded males and two included predominantly females without excluding males. The results are therefore only applicable to D-IBS in females. There have also been concerns about the association of Alosetron with ischaemic colitis and severe constipation which previously led to its withdrawal although it has now been re-introduced. Neither Tergaserod nor Alosetron have ever been available in the UK.

1.624 Analgesics.

Abdominal pain is widely accepted as one of the principle symptoms of irritable bowel syndrome and in many cases it is this pain that results in

patient consultation. [168] The role of both localised mucosal injury and inflammatory mediators and centrally mediated changes in pain perception in IBS has been discussed previously. In common with other chronic conditions in which pain is a significant feature such as osteoarthritis, it is important to consider the effects and side effects of both centrally and peripherally acting analgesic agents. Whilst many medications including antidepressants and anti-spasmodics may have an effect on pain they are not considered analgesics and are discussed separately.

Despite the fact that centrally and locally acting analgesics are frequently used in the management of patients with IBS, the evidence of efficacy is limited. Pregabalin has been shown to increase sensation thresholds in IBS patients with rectal hypersensitivity [169]. Asimadoline, a kappa-opioid agonist was also shown to improve pain in patients with IBS-D, but only in the overall cohort of IBS patients studied[170]. As such further evaluation is required and this compound is not currently used in clinical practice. Centrally acting opioid analgesia is often prescribed for pain in IBS with codeine, tramadol and morphine salts all used with little if any evidence of safety or efficacy in this patient group. The narcotic induced bowel syndrome, an under-diagnosed condition of increasing functional abdominal pain, as a result of inappropriate long-term opiate analgesia in patients with IBS remains a potential significant entity that clinicians need to be mindful of when using opiate analgesia in this patient group.[171] It is also of note that

in some studies the long term use of analgesics have been associated with an increased risk of IBS, but this is complicated by the increased incidence of somatic pain also associated with the condition. [172]

1.6.2.5 Other conventional medicines used in the treatment of IBS.

Two further drugs have recently been evaluated for efficacy in the treatment of IBS- C.

Linaclotide is a 14 amino acid, minimally absorbed peptide guanylate cyclase-C agonist that works by activation of the cystic fibrosis transmembrane conductance regulator to stimulate chloride secretion and thus increases gastrointestinal fluid secretion and colonic transit. Chey et al. in a study of 804 patients with IBS-C reported a responder rate of 33.7% vs. 13.9%

($p = <0.0001$) in the treatment and placebo groups respectively. [173] A responder was defined in accordance with the FDA, (Federal Drug Administration, USA) as a patient with an improvement of ≥ 30% from baseline in the average of worst daily abdominal pain score and an increase of ≥1 complete spontaneous bowel movement from baseline per week. A meta-analysis of three studies of Linaclotide with a total patient pool of 2024

patients with IBS-C using the same FDA approved outcome measure showed a pooled RR of 1.95 and NNT of 7. [174]

Lubiprostone is an orally active prostone that stimulates type-2 chloride channels (CIC-2) also increasing luminal fluid secretion and colonic transit. Drossman et al. in a combined analysis of two randomised controlled trials of lubiprostone with a total patient number of 1174 using the same FDA primary end-point reported a 17.9% vs. 10.1% (p = 0.001) in the treatment and placebo groups respectively. [175]

1.6.2.6 Antibiotics in the treatment of IBS.

The use of non-absorbable antibiotics in the treatment of IBS perhaps remains controversial. Several different systemic antibiotics were previously trialled including oxytetracycline and metronidazole, but the unwanted systemic affects and side effects have limited there use. Neomycin, and more recently rifaximin, are a more favourable option as they both are non-absorbable and as such should be less likely to produce any unwanted systemic effects. Pimental et al. found that neomycin in a randomised, double-blind controlled trial of 111 patients with IBS was significantly more effective at improving global symptoms severity 35% vs. 11.4% (p = <0.001)

and at normalising bowel habit, 35.3% vs. 13.9% (p = <0.001) than placebo.[176] However, it must be noted that 84% of patients in this study had positive lactulose breath tests (LBT) and that normalisation of the LBT after treatment predicted the clinical response in 75% of patients, hence these patients may have simply had SIBO without IBS and that treatment of the SIBO resulted in the symptom improvement. In a further study by Yang et al. they also found that normalisation of abnormal baseline LBT predicted clinical response in patients treated with rifaximin. They also found that repeated treatments with rifaximin remained efficacious whereas retreatment with neomycin was only effective in 25% of cases.

A further, landmark study by Pimental et al. of 1260 IBS patients treated with fourteen days of oral rifaximin versus placebo reported significant improvement in daily symptom severity (40.7% vs. 31.7%, p = <0.001), bloating (40.2% vs. 30.3%, p = <0.001) and average daily symptom scores (40.2% vs. 29.9%, p = <0.001). [177] However, whilst this study appears robust it does not report the frequency of abnormal baseline LBT or whether its normalisation predicts outcome. Although this could quite conceivably be a difference in study protocol, the principle author has utilised this argument for the presence of SIBO and improvement in LBT as a predictor of response in several previous studies and it arguably raises questions by its absence.

1.6.3 Psychological therapies in the treatment of IBS.

Multiple different psychological therapies are proposed as efficacious in IBS including psychoeducational support, mindfulness therapy and cognitive behavioural therapy (CBT). Of these the most widely used and researched is CBT which can take the form of a one-to-one clinician or nurse therapy, group therapy, distance or self-directed therapy. The cognitive behavioural model is based on the concept that events, emotions, thoughts, actions and physiological responses are all interlinked and in relation to IBS, the thoughts and interpretation of internal sensations and external events are of particular importance. The idea that 'this unpleasant sensation must mean that something is wrong' is thus interpreted as a potential threat leading to increased physical arousal anxiety and awareness of bodily sensations, which ultimately result in the original discomfort being experienced as more noxious and severe. [178] Multiple studies, of various design and quality report improvements in both physical symptoms and general well-being as a result of CBT, but as with other studies of medical interventions in IBS they suffer from significant methodological limitations. [179-183] However, a meta-analysis by Lachner et al including seventeen controlled studies reported an OR of 12 and NNT of 2 for psychological interventions in IBS.

1.6.4 Complementary medicine in the treatment of IBS.

A variety of alternative therapies are used for the treatment of patients with IBS including acupuncture, acupressure, ocular acupressure, various massage therapies, colonic irrigation, herbal medicine, Chinese herbal medicine, homeopathy and hypnotherapy. The evidence base for alternative medicines in the treatment of IBS is extremely limited. Both acupuncture and hypnotherapy have been the subject of Cochrane reviews and in both cases the reviewers concluded that there were insufficient studies of acceptable quality to draw any conclusions.[184, 185] Often, in clinical practice, patients report beneficial effects of a variety of alternative and complementary treatments but this may simply reflect a placebo response. The relative lack of efficacious conventional treatment may result in desperation in some patients who are 'willing to try anything' and ultimately, sometimes in desperation, turn to alternative/complementary treatments. Whilst, the vast majority of complementary treatments involve no risk to the patient, reasonable caution should be recommended with any ingested or invasive procedures. Conventional medicine endeavours to practice evidence based medicine but this is sometimes not the case. It may not be considered reasonable or ethical for conventional health practitioners to recommend

complementary therapies but to disregard or undermine them with a lack of an efficacious conventional alternative may do patients a disservice.

It is not the intention of the author to give a comprehensive or exhaustive review of differing treatment modalities and the associated evidence of efficacy for them and this does not fall within the scope of this thesis. It is worth noting that a large proportion of the clinical studies for IBS treatments are limited not only by the heterogeneity of the condition itself but also the lack of use or availability of standardised or validated instruments or measures of outcome. In addition, this lack of good scientific practice and methodological approach serves only to compound these problems. The vast majority of therapies available either lack sufficient efficacy or have too frequent side effects to make them tolerable or acceptable to patients.

Table 1: Diagnostic classification systems for the diagnosis of IBS.

i, Manning Criteria
Increased frequency of defecation with the onset of pain **Stools more 'loose' with the onset of pain** **Abdominal pain eased by defecation** **Abdominal Distension** **Passage of Mucus** **Feeling of incomplete evacuation**

ii, Rome I Criteria
Minimum of 3 months continuous or recurrent symptoms:
Abdominal pain/discomfort* which is: **Relieved by defecation (and/or)** **Associated with a change in stool frequency and/or consistency**
And 2 or more of the following (occurring at least 1 day in 4 on average)
Altered stool frequency **Altered stool consistency (lumpy/hard/watery/mushy/loose)** **Difficulty defecating (straining/urgency or feeling of incomplete evacuation)** **Passage of mucus** **Abdominal bloating/distension**

iii, Rome II Criteria
12 weeks or greater of abdominal pain/discomfort* in the last 12 months (does not need to be consecutive)
and 2 of the following:
Pain/discomfort relieved by defecation Onset of pain/discomfort* associated with change in stool frequency Onset of pain/discomfort* associated with a change in consistency of stool
Other symptoms not required but supportive of a diagnosis of IBS
Abnormal frequency of stool Abnormal stool consistency (lumpy/hard/mushy/loose/watery) Difficulty passing stool (straining/urgency or feeling of incomplete evacuation Passage of mucus Abdominal bloating/distension

iv, Rome III Criteria
3 months or greater of abdominal pain/discomfort in the last 6 months (does not need to be consecutive)
Associated with 2 or more of the following for at least 25% of the time:
Pain/discomfort* relieved by defecation Onset of pain/discomfort *associated with change in stool frequency Onset of pain/discomfort* associated with a change in consistency of stool
No Evidence of any inflammatory anatomic, metabolic or neoplastic process that explains the symptoms

*'discomfort' is defined as an uncomfortable sensation not described as pain

Description of the various diagnostic classification systems used in the idetifiaction and diagnosis of patients with IBS. (adapted from): Adapted with permission for reuse from; Saito et al. 2003 [12] and Romecriteria.org

Table 2: Rome III classification of IBS sub-type according to predominant stool form.

IBS Subtype	
IBS – C (constipation)	Hard or lumpy stools > 25% of the time *and* Loose (mushy) or watery stools < 25% of the time
IBS – D (diarrhoea)	Loose (mushy) or watery stools > 25% of the time *and* Hard or lumpy stools < 25% of the time
IBS –M (mixed)	Hard or lumpy stools > 25% of the time *and* Loose (mushy) or watery stools > 25% of the time
IBS – U (un-subtyped)	Insufficient abnormality of stool consistency to meet the criteria for IBS- C/D/M

In the absence of anti-diarrheals or laxatives.

Hard or lumpy stools: Bristol Stool Form Scale 1-2
Loose (mushy) or watery stools: Bristol Stool Form Scale 6-7

Description of IBS subtype classification according to predominant stool type.

Table 2 adapted with permission from Longstreth et al 2006[5]

Table 3: Summary of studies of the prevalence of IBS using different methodology and diagnostic criteria.

Author	Date of Publication (date of survey)	Population studied / method	Diagnostic criteria	Population/ Response rate (sample size (%))	Male/Female ratio	Prevalence (%)
Gwee KA et al [22]	2004 (1998-2002)	Face-to-face survey of random population sample (Singapore)	Manning (3) Rome I Rome II	2276/3000 (75.8%)	1:1.3	11 10.4 8.6
Hillila MT et al [8]	2004 (2001)	Postal Survey (Finland)	Manning (2) Manning (3) Rome I Rome II	3650/5000 (73%)	1:1.2	16.2 9.7 5.6 5.1
Wilson S et al [15]	2004	Postal survey of patients from group 4 local GP practices (UK)	Rome II	4807/8386 (57.3%)	2.1:1	10.5
Andrews EB et al [23]	2005 (2001-2)	Internet/ e-survey of general population sample (USA)	Rome II	25986/31829 (82%)	1:1.8	6.6
Okeke EN et al [24]	2005	Questionnaire to sample of university students (abstract only) (Nigeria)	Rome II	330	1:1.8	26.1
Vandvik PO et al [25]	2006 (2001)	Questionnaire administered to sample of general population at local mobile unit and by post (Norway	Rome II	4622/11078 (42%)	1:1.9	8.4
Sherber AD et al [34]	2007	Telephone interview	Rome II Rome III	1000/1221 (81.9%)	1:2 1:1.4	2.9 11.4

Author	Date of Publication (date of survey)	Population studied / method	Diagnostic criteria	Population/ Response rate (sample size (%))	Male/Female ratio	Prevalence (%)
Katsinelos P et al [26]	2009 (2004-7)	Questionnaire from a primary care clinician for 'non-patients' attending a community hospital (Greece)	Rome II	2397/3112 (67.1%)	1:3	15.7
Perveen I et al [27]	2009 (2004/5)	Door step survey of general population sample (Bangladesh)	Rome II	1503/- (97.2%)	1:1.36	7.7
Valerio-Urena J et al [29]	2010	Face-to-face interview of random general population sample (Mexico)	Rome II	459	1:2.5	16.9
Zhao Y et al [30]	2010 (2007/8)	Self-administered questionnaire of general population sample (completed at home or in regional office) (China)	Rome II	16091/18000 (89.4%)	1:1.2	4.6
Khademolhosseini F et al [32]	2011 (2004)	Postal Survey (Iran)	Rome II	1978/3600 (54.9%)	1:1.7	10.9
Gulewitsch MD et al [31]	2011 (2009)	Internet/ e-survey of university students (Germany)	Rome III	2196/2399 (91.6%)	1:1.4	19.4
Makharia GK [33]	2011 (2008/9)	Door to door Survey (India)	Rome III	4767	1:1.5	4
Dong YY et al [28]	2010 (2009)	Survey of university students (North China)	Rome III	2126/2500 (85%)	1:1.8	7.9
Nam et al [29]	2010 (2007)	Questionnaire given to participants in health screening programme (Korea)	Rome II	4296	(OR Female 1.33)	8.2

Author	Date of Publication (date of survey)	Population studied / method	Diagnostic criteria	Population/ Response rate (sample size (%))	Male/Female ratio	Prevalence (%)
Valerio-Urena J et al [29]	2010	Face-to-face interview of random general population sample (Mexico)	Rome II	459	1:2.5	16.9
Zhao Y et al [30]	2010 (2007/8)	Self-administered questionnaire of general population sample (completed at home or in regional office) (China)	Rome II	16091/18000 (89.4%)	1:1.2	4.6
Khademolhosseini F et al [32]	2011 (2004)	Postal Survey (Iran)	Rome II	1978/3600 (54.9%)	1:1.7	10.9
Gulewitsch MD et al [31]	2011 (2009)	Internet/ e-survey of university students (Germany)	Rome III	2196/2399 (91.6%)	1:1.4	19.4
Makharia GK [33]	2011 (2008/9)	Door to door Survey (India)	Rome III	4767	1:1.5	4

Summary of previous studies of prevalence of IBS in different population groups. Specific information given about methodology used, diagnostic criteria applied, response rates, male to female prevalence ratio and overall prevalence.

Chapter 2:

The Intestinal mucosa, luminal microbiota, probiotics and gut function.

2.1 The intestinal mucosa

The human gut has a mucosa that is comprised of a variety of different cellular and non-cellular components with a surface area that is approximately 400 m^2.[186] In its simplest form it can be considered a simple physical barrier between the gut lumen and the host. However, this oversimplified description fails woefully to do justice the complex integrated functions of both the gastrointestinal mucosa and its interaction with the luminal contents. Whilst the mucosa itself acts as a physical barrier it must also facilitate the passive and active transfer for nutrients from the luminal contents to the host. In addition it has a vital role in the prevention of microbial invasion and the development of immunocompetance and tolerance. [187-189]

The mucosa itself consists of a single layer of columnar cells whose apical surface are covered in microvilli and a glycocalyx mucous layer which together form the 'brush border'.[186] Interspersed between the columnar cells are follicle associated epithelium (FAE), a highly specialise epithelium which plays a key role in the mucosal immune function.[190] The FAE is found overlying areas of the mucosa associated lymphoid tissue (MALT) throughout the GI tract.[190] The FAE and Peyer's patches contain important antigen presenting cells including M-cells and immunoregulatory cells and phagocytic cells. [190] Within the mucosa itself intra-epithelial lymphocytes are found and in the underlying lamina propria there are a large number of

terminally differentiated B-cells, T-cells and dendritic cells.[191] In certain areas of the distal small bowel, where the luminal content contains high numbers of micro-organisms, the FAE is found in more organised structures caller ‘Peyer’s patches’. Together the FAE, MALT, Peyer’s patches, lymphoid follicles, intra-epithelial lymphocytes, mesenteric lymph nodes and lamina propria contain the largest aggregation of immune-competent immune cells within the human body, and is collectively known as the gut associated lymphoid tissue (GALT).[191, 192]

The antigen presenting phagocytic and immunoregulatory cells of the mucosal immune system allow the production of a rapid, selective and potent immune response to the threat of invasion by harmful pathogenic bacteria from within the gut lumen.[190, 193] These complex interactions also serve to develop the ability of the immune system to differentiate between harmful and commensal bacteria, nutrients and food antigens. In addition, the interactions between T-cells and MHC-II expressing antigen presenting cells, leads to modulation of particular anti-inflammatory pathways to down regulate the immune response and favour tolerance. [194]These processes are vital so that the powerful immunogenicity to harmful microbiota does not result in inappropriate immune activation to commensal bacteria or food antigens. [187, 193] The symbiosis that results between the host and the luminal microbiota as well as being regulated by a complex network of physiological and immune factors is also influenced by other host and – non-

host factors including age, antibiotic use, established immune competency and the gut flora itself. [195] Throughout the life of the host, the commensal bacteria of the bowel are involved in instructing the immune system and are ultimately responsible for the presence of inflammatory and immune cells within the healthy gut. This so-called physiological, or controlled inflammation, refers to the described presence of inflammatory and immune cells within the mucosa and sub-mucosa of the gastrointestinal tract, reflecting the degree of mucosal immune accommodation that is a requirement to normal, healthy gut function.[196] At the same time it must also be noted that the healthy commensal bacteria, whilst living in symbiosis with the host when contained with the gut lumen, can if they gain access to another body cavity (e.g by perforation of the viscera or by entry into the vascular compartment) become potentially fatal to the host.

2.2 The intestinal microbiota and its role in the development and function of the gastro-intestinal tract.

The human gastrointestinal microbiota is a complex ecosystem; containing between 300-500 different known species of microorganism [197] and likely many more uncharacterised or unknown species, more than ten times the number of eukaryotic cells of the host[198] and makes up approximately 55% of the total faecal mass[199]. Direct culture of the

intestinal microbiota is technically difficult with between 40 and 80% of the observed microbial count being unrecoverable through conventional culture techniques.[200, 201] In addition, differing regions of the GI tract have markedly different environmental conditions resulting in differing microbial populations and as such stool cultures may only reflect the microbial population of the very distal colon and rectum. Recent advanced gene amplification techniques using 16s rRNA and rDNA sequences are increasingly used to investigate the composition of the human microbiota. These techniques have shown that there are at least 82 different microbial species and 284 individual clones present in the adult-male faecal sample. Furthermore, of the 284 clones identified 76% did not correspond to genetic sequences available in public library resources and are potentially hitherto unknown species, that may be unique to the human intestinal microbiome. [200]

The gastro-intestinal microbiota are not simply the fortunate beneficiaries of the complex and tolerant mucosal immune system of the host, but rather an integral part of the development and function of the mucosa, physiology and immune system of the human gut. Evidence for these interactions and the role of the microbiota in normal gut function and disease, comes from work in animal models and particularly work in germ-free animals.

Germ-free rodents are instrumental in our understanding of mucosal microbibiota interaction. However they seem to have an enlarged caecum which resolves on the introduction of normal commensal flora suggesting that the gut microbiota influence the motility of the large bowel. [202] Further evidence of the role of the microbiome in gut motility is suggested by the failure of differentiation of the serotonin (5-HT, 5 hydroxytryptamine) secreting enteroendocrine cells of the bowel in a germ-free environment, leading to reduced colonic motility.[203, 204] This abnormal motility is likely to be a result of these and perhaps other yet unknown factors. Short chain fatty acids (SCFA) are produced as a result of microbial fermentation and are essential for the proliferation and differentiation of gut epithelial cells.[205, 206] In germ-free mice, the absence of the trophic effect of the SCFAs leads to a decreased turnover of cells with the mucosal crypts and less total number of crypts.[205] There is also evidence to suggest that these SCFAs are also the principle source of energy for the colonic enterocytes and thus the microbial fermentation is essential for the absorption of water, sodium and other essential minerals including calcium and magnesium.[207, 208] Further potential support for the importance of the microbiota in nutrition is seen in the clinical phenonoma of the development of a mild coagulopathy, that can result from the use of broad spectrum antibiotics suggesting the importance of bacterial synthesis of vitamin K_2 (menaquinone).[209]

The role of the gut microbiota in the development of the host immune system seems to be of particular importance in early life. The interactions

between these microorganisms and the gut immune cells are essential in the development of both immune competence, memory and tolerance of the complicated immunoregulatory systems of the gut.[210] In germ free rodents the GALT is developmentally deficient. However, this can be reversed by early introduction of microbes into the gut lumen in the neonatal period, but not in adult life.[210] The microbiota continue to interact both local and systemically with the host immune system and can lead to both an immunoregulatory effect, resulting in inflammation refractory states, or propagate an immune response' resulting in an exaggerated immune response.[211] Some species of luminal commensal bacteria, such as lactobacillus acidophyllus, are also known to directly inhibit the adhesion of pathogenic bacteria such as salmonella Typhimurium and Escherica coli.[212] The interactions of the microbiota within the host immune system promote an immune-tolerance that provides a considerable advantage to the micro-organism in a food rich environment with optimal living conditions. In addition, they can have a more direct influence on the luminal environment, with some organisms producing enyzmes that regulate the production of essential nutrients by the host enterocytes and in so doing so produce an optimal environment in which it may prosper.[213]

2.3 The Role of intestinal microbiota in pathogenesis of IBS and other disease.

The scale and complexity of interactions of the commensal and pathogenic microbiota of the human GI tract and the mucosa and immune system of the host alone, would implicate the potential for their involvement in the development of disease of GI tract. The role of the gut microbiota in IBD is not fully established, however, in experimental genetic models of inflammatory disease colonisation of the gut lumen is required for the experimental colitis to develop.[214] Human studies show differences in the composition of the gut microbiome in patients with active ulcerative colitis and active and inactive Crohn's disease when compared to controls.[215] The importance of this finding and the nature of the relationship (cause or effect) however is yet unclear. Furthermore, the microbiota of relatives of Crohn's patients has also been shown to be different from controls.[216] In paediatric coeliac disease there is again a differing profile of gut bacteria compared to normal subjects.[217]The role of the gut microbiota in disease pathogenesis is not limited to those that affect the GI tract.

In experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis, the gut microbiota are important in the disease induction by virtue of their stimulatory effect on pro-inflammatory T-cell

responses.[218] In neonates, differences in the gut microbiota and colonisation with Bacteriodes fragilis correlates with a high asthma predictive index and the development of asthma and atopy.[219] In non-alcoholic fatty liver disease, (NAFLD) small bowel bacterial overgrowth with gram negative organisms in animal models is linked to increased endogenous alcohol production, choline deficiency and insulin resistance, all factors that have been implicated in its pathogenesis. The potential of specific functions of the gut microbiome in the development and pathogenesis of various diseases is keenly explored but its discussion is outside the scope of this thesis.

Knowledge of the pathogenic mechanisms that lead to the development of IBS is still expanding, but it is becoming clear that the gut microbiota potentially are a key player in its development and in the on-going temporal variability of symptoms. Key quantitative and qualitative differences in the gut microbiome of IBS patients have been demonstrated using gene cloning and sequencing techniques. The faecal microbiota of patients with IBS has been shown both to differ from that of healthy controls [220, 221] and to have an increased temporal instability.[221] Specific differences in the predominant species of commensal microorganisms in IBS patients is reported, but the importance is not clearly understood and some of the results are conflicting with both increased [222] and decreased [223] microbiotal diversity reported. Increased numbers of firmicutes and bacteroides together with large decreases in the number of bifidobacterium

and faecalibacterium groups are reported.[223] Lower levels of Lactobacilli sp. are found in IBS-D,[224] and differences in the proportions of the predominant Clostridium coccoides –E rectal species in both IBS-D and IBS-C.[221] Further differences with decreased levels of clostiridum coccoides and bifidobacterium catenulatum group together with increased levels of ruminococcus productus-C coccoides in all IBS sub-groups has also been described.[224] Many other observed differences have been noted but the relevance of these findings has not been established.

Small intestinal bacterial overgrowth (SIBO) has been long proposed as a potential pathological mechanism for IBS but this remains controversial. Hydrogen and methane gases are both a bi-product of bacterial fermentation and higher levels of these are reported in patients with IBS. [225] In research and clinical practice the hydrogen breath test using either lactulose or sucrose as an orally delivered substrate are used to diagnose SIBO. However, whilst some authors report high levels of up to 78% of SIBO prevalence within IBS patient cohorts, [226] others report no significant difference in SIBO between IBS patients and healthy controls. [227] Further support for the SIBO hypothesis is levied at the improvement seen in IBS symptoms in trials using broad spectrum non-absorbable antibiotics, [176, 177, 226] although much of this work comes from the same research group and again has been criticised for its methodology. The presence of increased levels of bacterial fermentation and methanogenic

colonic bacteria is fairly universally accepted, but the presence of SIBO in the context of IBS continues to split opinion.

The effects of bacterial fermentation on the gut motility and the potential role for abnormal fermentation patterns in IBS patients, can be understood when the utility of the products of bacterial fermentation are considered. In the human colon a large quantity of unabsorbed carbohydrate is delivered to the large colon where it is fermented by the luminal bacteria to yield SCFAs. The SCFAs are utilised as the main source of energy for colonocytes and are absorbed by both non-ionic diffusion and by active transport, mediated through a sodium coupled transporter leading to active sodium and water influx.[207, 228] In addition the SCFAs provide the energy source for sodium absorption via the sodium-proton exchange. [207] Changes in the gut microbiota may lead to decreased production of SCFAs and loss of this stimulatory effect coupled with the osmotic effect of increased non-absorbed carbohydrate in the gut lumen can result in significant diarrhoea.[229] Patients with IBS-D have a different composition of colonic SCFAs, with relatively higher levels of n-butyrate and less acetate as a result of differing colonic bacteria.[207] Specific overgrowth of methanogenic bacteria in the colon is exclusively associated with IBS-C and it seems like the methane itself may have a direct effect on gut motility.[230] The presence of hydrogen consuming bacteria in the gut lumen is also a

factor and reduced numbers of these bacteria in IBS patients may result in some of the observed abnormal gas handling.[225]

It is proposed that the pathogenesis of IBS may result from exposure to differing microbiotal pools in early childhood and in particular the relative lack of exposure to pathogenic gut bacteria. [45] The ‘hygiene hypothesis’ is certainly plausible given the complex interactions between the gut microbiome and the host.

The development and employment of new gene sequencing techniques is rapidly expanding the available knowledge of the gut microbiota and will undoubtedly lead to a greater understanding of its role in the development, function and disease of the human GI tract.

2.4 Probiotics in the treatment of IBS

2.4.1 Mechanisms of action of probiotics

The importance of the gut microbiome in the development and maintenance of normal gut physiology, motility, immune-tolerance and competence has already been established. The probiotic concept differs in that it refers to the utilisation of specific strains of microorganism and their properties that, if ingested, will potentially enhance normal physiological

function, prevent or treat disease, alleviate symptoms or restore normal function.

It is widely accepted that the first observations of the positive health benefits of live microorganisms were made by Professor Élie Metchnikoff; a Russian scientist working at the Pasteur Institute who went to on to become the Nobel Laureate. He observed that the fermented milk products consumed in rural communities in Bulgaria contained lactic acid producing bacteria that may contribute to the extended life expectancy of those communities in which they were consumed. [231] This microorganism later became known as Bulgarian bacillus and ultimately *lactobacillus bulgaricus sp.* and is perhaps the first documented probiotic. In 1917, following an outbreak of shigella, German professor Alfred Nissle isolated the bacterial strain that later became known as "*Eschericia coli" 1917* from the faeces of a soldier not affected by the outbreak. In a time when antibiotics were not readily available this bacterial strain became a common treatment for infective gastroenteritis. Despite the fact that the concept of probiotics has been around for more than a century, our understandings of the mechanisms of action are still limited and even the definition as to what constitutes a probiotic is not universally agreed upon. However, The World Health Organisation (WHO) definition of what constitutes a probiotic is now generally accepted it states that a probiotic is :

'A live bacterial product that if taken in sufficient quantity results in a positive health benefit'

A principle problem of describing the mechanisms of actions of a probiotic, is that the individual mechanisms may be unique to a particular species and/or strain of micro-organism, as well as being relevant only to a particular host or disease state. As such, this description will concentrate on mechanisms of action that are relevant only in the context of IBS and will also make the assumption that low grade inflammatory responses, altered mucosal permeability, antigenic hypersensitivity, visceral hypersensitivity and altered motility all contribute to the pathogenesis and symptoms of IBS.

Transient *Trichella spiralis* infection in murine models can lead to altered visceral perception and dysfunctional motility.[232] In a similar model, ingestion of *Lactobacillus paracasei NCC2461* results in the reversal of muscle hypercontractility; visceral hypersensitivity and decreased cyclooxygenase 2 (COX-2) expression. [233] This result provides evidence of the potential for a probiotic to have an effect either directly or indirectly at the neuromuscular junction, resulting in a change in motility and also have anti-inflammatory and anti-nociceptive effects. In the same study several different probiotics were studies but *L. paracasei NCC2461* was the only one to produce these results, supporting the idea that probiotic effects are strain specific. A further study by Bar et el. examined the effect of exposure to *Escherichia Coli Nissle 1917* on isolated human colonic muscle fibres in an vitro organ bath and found increased fibre contractility, supporting the concept that probiotics can influence gut contractility and motility. [234]

Indirect evidence of the clinical effects of probiotic on gut motility is provided by Johansson et al. in their study in which the probiotic *Lactobacillus plantarum DSM 8943 (299v)* was given to a group of normal human volunteers. The probiotic produced a change in luminal colonic pH and thus a change in the environmental conditions to favour the growth of particular bacteria. The outcome was an increase in the faecal quantity of bifidobacteria and lactobacilli as well as improved motility, increased stool volume and decreased flatus. [235]

In a rodent model of stress induced colonic dysfunction by Eutamene et al. the observed increased permeability and visceral hypersensitivity are both improved by *L. paracasei NCC2461.* [136] The ability of probiotics to improve visceral hypersensitivity is further supported by the observations of sustained increased expression of opioid and cannabinoid receptor mRNA, seen with ingestions of *lactobacillus acidophilus NCFM* [236] and a separate murine model of antibiotic induced visceral hypersensitivity alleviated by *L. paracasei NCC 246*. [237] Probiotics may also have the ability to moderate intestinal antigenic responses and thus reduce inappropriate inflammatory responses to intestinal luminal content. *Lactobacillus rhamnosis GG* and *Bifidobacteria animalis MB5* have both been shown to increase expansion of anti-inflammatory T-cell populations and increase IL-10 secretion and thus decrease intestinal allergenic response in a rodent model. [238]

The evidence for different mechanisms of action of probiotics within the intestinal lumen is extensive and much of it may be relevant to their efficacy in IBS. As discussed these may include actions on the neuromuscular junctions and motility, visceral hypersensitivity, inflammatory and allergenic responses and mucosal permeability. In addition to their direct actions, probiotics also have an effect on the luminal environment and as such may also result in favourable growing conditions for certain groups of bacteria. These bacteria themselves may have a probiotic effect and thus the original positive effect may be amplified or added to. Perhaps the only certainty that can be gleaned from the evidence is the likelihood that all mechanisms of actions may be specific to the species and perhaps even the strain of probiotic.

It is difficult to describe particular characteristics of individual probiotic species and strains that could be classified as ideal and/or required of a probiotic to be used to treated patients with IBS. However, clearly any such probiotic must be delivered to the recipient in such a way that the individual bacteria are live and viable (able to divide and colonise the gastrointestinal lumen of the recipient). They must also be able to survive transit through the arguable hostile environment of the upper gastrointestinal tract and lastly be able to adhere to, and colonise, the colonic mucosa without resulting in an unfavourable immune/inflammatory responses. Whether long term colonisation and/or persistence with the colon is desirable is debatable as periodic dosing would overcome such a limitation. Moreover, if the goal of

therapy with a probiotic is to return the colonic microbiota to a favourable composition which doesn't perpetuate the symptoms of IBS then long term colonisation may not be required or even desirable.

Until the pathological mechanisms of IBS are fully understood it is difficult to specify more detailed characteristics that would be desirable in a probiotic IBS therapy. Whether the anti-inflammatory properties, anti or pro-motility characteristics, local or distantly acting immunogenic or modulating characteristics that a variety of different bacterial strains have been demonstrated to poses are yet to be clearly established.

The probiotic preparation Symprove is a multi-strain probiotic containing 4 individual strains of bacteria (*Lactobacillus plantarum NCIMB 30173, Lactobacillus rhamnosus NCIMB 30174, Lactobacillus acidophilus NCIMB 30175, Enterococcus faecium/durans NCIMB 30176*). Historically, the product was developed as a way of preserving germinated grain as a high quality agricultural feed. The bacterial strains were used in a cascade sequence to reduce the pH of germinated 'wet' grain from pH 7 to pH 4 and in so doing improved the shelf life of the 'wet' grain from 10 days to 4 months. Subsequent observations by breeders and veterinary professional of livestock fed with the treated grain demonstrated unexpected results. Whilst much of this information is from observational work by the developers and/or anecdotal evidence, these observations included, reduced dependence on antibiotics, increased appetite, improved lean juvenile growth

rates and decreased mortality. Eventually realising that the observed benefits were as a result of the contents of the ‘pickling liquor’ and not the germinated grain itself the developers set about understanding the pickling process itself.

Ultimately, the process of grain pickling was reverse engineered so that the germinated grain was utilised to provide the growing substrate for the ‘probiotic’ bacteria. The resultant bacterial product was then used as a veterinary supplement in ‘small animals’ for numerous years to treat gastrointestinal disturbances before the final refinement of the product for use in humans.

Symprove been commercially available for several years as a ‘health food supplement’ used by consumers for a variety of indications. However, until this time there has been no formal assessment of efficacy of the product in the treatment of any gastrointestinal conditions in the human population. Furthermore, no in-vitro studies have been undertaken to ascertain any of the individual characteristics and properties of the individual microbial strains that may account for the presumed efficacy of the product in the treatment of gastro-intestinal disturbances.

2.4.2 Current evidence of the efficacy or probiotics in the treatment of IBS and their role in clinical practice.

The evidence for the efficacy of probiotic therapy in the treatment of patients with irritable bowel syndrome suffers from the same limitations common to all IBS studies. As previously discussed these include the considerable heterogeneity of the disorder itself, differing sub-groups characteristics, changing classifications of the disorder and the lack of a positive diagnostic test. In addition, there is no clearly established definition of what constitutes a clinical response or how to define a patient responder in terms of the individual symptoms of IBS, as well as global symptom improvement. Recent guidelines produced by both the Federal Drug Authority (FDA) in 2010 and earlier guidelines produced by the European Agency for the Evaluation of Medicines (EMEA) in 2003, have sought to address some of these problems and standardise both inclusion criteria and outcome measures for such trials. However, both sets of guidelines differ considerably in their recommendations and a recent statement from the EMEA working group highlighting the need for comprehensive international guidelines to facilitate international collaboration and comparisons with and between research groups.

A particular problem that appears endemic amongst trials of probiotics in IBS is the apparent poor quality and methodological limitations of the studies themselves. The vast majority of probiotic and live microbial products are considered as foods, food supplements and health foods and as such, until very recently, have not been subjected to the same stringent

regulatory controls as medicines. It is quite possible that this fact alone coupled with the evidence that larger, more robust trials are significantly more difficult and costly to undertake; has resulted in the prevalence of poor quality studies within this field. Whilst arguably there is no direct evidence to support this belief, it is one that is held by many within the academic and scientific community.

Of the hundreds of original studies looking at the safety and efficacy of probiotics in the treatment of IBS, there are in fact very few that conform to the standardised randomised placebo controlled trial format and of these even fewer could be considered of sufficiently high quality. Recently there have been several meta-analyses and systematic reviews that have attempted to address these problems and answer the question of whether probiotics in general and/or specific probiotics, have sufficient evidence of efficacy in the treatment of IBS. The results of these meta-analyses will be considered collectively rather than as individual studies themselves.

Of the clinical studies analysed by the authors, very few were actually deemed of sufficient quality to include in the meta-analyses. McFarland et al, after assessing study quality using the Linde Internal Validity Scale (LIVS) identified a total of fourteen trials for inclusion in their meta-analysis.[32] Similarly, Moayyedi et al., identified eighteen trials and

Hoveyda et al. fourteen trials using similar trial quality rating tools. [239, 240] Table 4 summarises the study size, duration, disease sub-types probiotic strain(s), outcome measures and primary endpoints and other significant results from the trials included in all three meta-analyses. Understandably the majority of the trials feature in all three meta-analyses, but the full papers of some could not be accessed using on-line sources and search engines and so these studies have not been included in the table.

Hoveyda et al. concluded that overall there was modest improvement in global symptoms after treatment with probiotics when compared to placebo. The overall OR for improvement in global symptoms reported as dichotomous data was 1.6 in favour of probiotics (95% CI 1.2 to 2.2); heterogeneity I^2 = 28% (figure 1); and for continuous data the standardised mean difference (SMD) was 0.23 (85%CI 0.07 to 0.38);heterogeneity I^2 = 0%. [239] (figure 2). McFarland et al. reported a pooled RR of 0.77 (85% CI 0.62 to 0.94). NNT 7.3; for less global symptoms after treatment with probiotics compared to placebo using a random effect model, heterogeneity X^2 = 41.0 d.f. [32] (figure 3). Moayyedi et al. reported a reduction in global symptoms with probiotics over placebo in studies reporting a dichotomous outcome with a pooled RR 0.71 (95% CI 0.57 to 0.88), NNT 4, heterogeneity X^2 28.3 d.f. (figure 4) and in those using continuous data an SMD of -0.34 (95% CI -0.60 to -0.07) heterogeneity X^2 = 67.04 d.f. (figure 5).

Abdominal pain scores were also shown to improve after treatment with probiotics over placebo. Moayyedi et al. reported an improvement in abdominal pain with an SMD of -0.51 (95 CI -0.91 to -0.09), heterogeneity X^2 = 61.08 d.f.,[240] Hoveyda et al. in studies using dichotomous data with an of OR 2.88 (95% CI 1.84 to 4.50), heterogeneity I^2 = 1%; [239] (figure 6) but no benefit in those using continuous data, SMD 0.05 (95% CI - 0.09 to 0.19), heterogeneity I^2 51%; and McFarland et al. a pooled RR of 0.78 (95% CI 0.69 – 0.88), heterogeneity X^2 = 36.6 d.f. (figure 7). The number of studies reporting on other associated IBS symptoms were significantly fewer but Hoveyda et al. reported a significant improvement in bloating with studies using dichotomous data (four studies) OR 1.75 (95% CI 1.03 to 2.96), I^2 = 0%; but no difference in those reporting bloating using continuous data, SMD 0.05 (95% CI -0.10 to 0.21) I^2 = 8% (figure 8). [239]

All three meta-analyses reported similar significant heterogeneity between studies but a modestly beneficial effect of probiotics on global symptom and abdominal pain. The similar findings from the separate meta-analyses are not surprising in that they all review a similar set of studies. Furthermore, it is also interesting to note that Mcfarland et al. found that larger studies reported a stronger positive effect of probiotics with Moayyedi et al. reporting similar effect in studies of higher methodological quality.[32, 240] All three sets of authors rightly stress that the significant heterogeneity, frequent lack of ITT analyses and overall relatively poor quality of included

studies seriously limit the validity of the meta-analyses. Even more importantly it must be considered that at least 60 individual probiotic strains and 19 different products were considered in the meta-analyses and therefore it is impossible to draw any unifying conclusions for probiotics as a whole. Perhaps the only reasonable conclusions to make are that of the probiotics studies for the treatment of IBS, the majority seem to have some modest efficacy and good safety profiles and that the efficacy of each probiotic strain and preparation must be considered to apply only to that species and/or strain alone. Finally, the results should not be extrapolated to other strains or species and cannot be applied to 'probiotic' preparations as a 'class effect'.

Table 4: Summary of randomised double blind placebo controlled trials of the efficacy of probiotics in the treatment of irritable bowel syndrome.

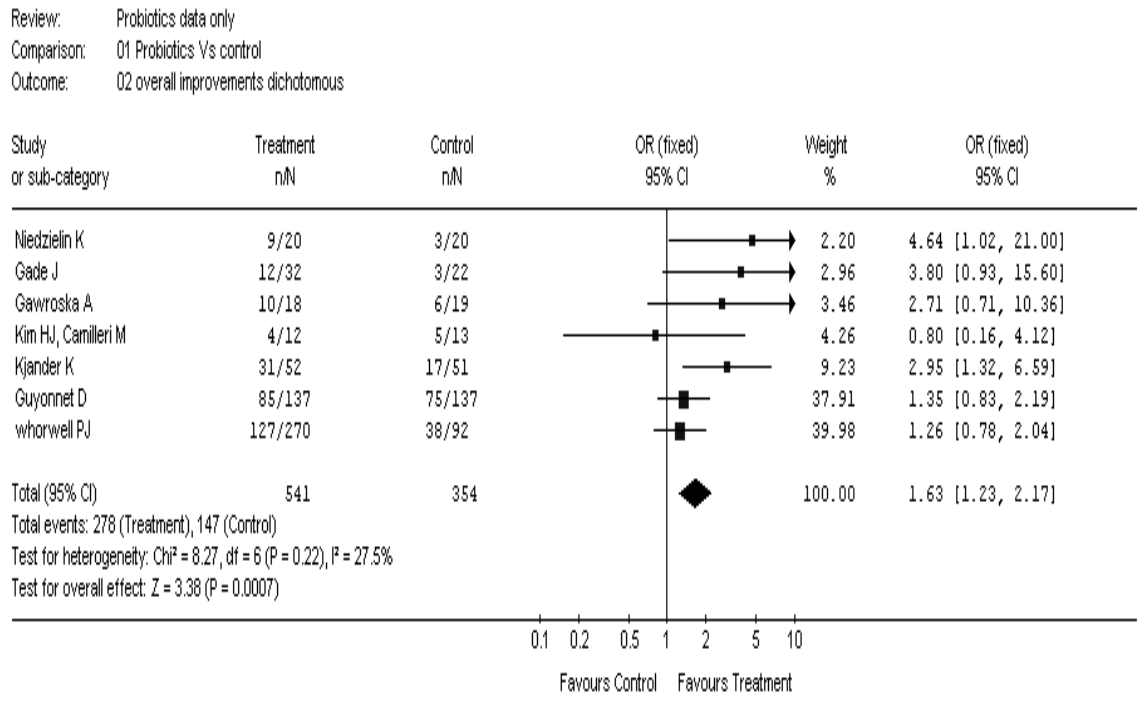
Author (Year published)	Participants (age Range)	Diagnostic criteria and sub-group	Length of treatment	Probiotic used	Outcome measures	Results of primary outcome and other important results
Enck [28] (2008)	297 (19-76)	Primary care classification	weeks	*Escherichia coli DSM 17252* and *Enterococcus faecalis DSM16440*	No prior P-EP specified. Study a re-analysis of previous work to give P-EP as improvement in abdominal pain and global symptom score (computed). Responder defined as at least 50% improvement.	P =EP: Global symptom score, responders 68.5% probiotic vs. 37.8% placebo ($p < 0.001$). Abdominal pain responders 72.5% probiotic vs. 44.6% placebo ($p < 0.001$). NNT 3.59.
Guyonnet [241] (2007)	274 (20-65)	Rome II IBS-C	6 weeks	*Bifidobacterium animalis DN1730101 + S. thermophiles and Lactobacillus bulgaricus*	Functional digestive disorders quality of life (FDDQL) P-EP – discomfort HRQoL (health related) at week 3, responder defined as improvement of >10% from baseline score S-EPs other HRQoL dimensions. Bloating abdominal pain stool characteristics and global digestive symptoms week 3 and 6 (Likert scales).	HRQoL discomfort responders higher 65.2 vs. 47.7%, $p < 0.005$ (P-EP). Decrease bloating score 0.56 ± (s.d.)1.01 vs. 0.31 ± 0.87, $p <0.03$. No difference in HRQoL dimension actual score or symptom severity scores other than bloating at week 3 or 6.
Gawronska [242] (2007)	104 (37 IBS) (6-16)	Rome II (FAPD)	4 weeks	*Lactobacillus rhamnosus GG*	P-EP: no pain (a relaxed face on 'faces pain score'). S-EPs: improvement of at least 2 'face scores' from baseline, self-reported pain severity, frequency of self-reported pain and use of medication for abdominal pain.	P-EP: Overall study population: 25% probiotic vs. 9.6% placebo (relative benefit RB 2.6, NNT 7). IBS group: 33% probiotic vs. 5% placebo, RB 6.3, NNT 4)
Whorwell [243] (2006)	362 (19-69)	Rome II	4 weeks	*Bifidobacteria infantis 35624* (Dose ranging study: 1×10^6, 1×10^8 and 1×10^{10} vs. placebo)	P-EP comparison of daily abdominal pain score at week 4. S-EPs comparison of bloating/distension, incomplete evacuation straining, passage of gas urgency and overall self-reported global symptom assessment (SGA).	P-EP: improvement in abdominal pain vs. placebo, -0.89 vs. -0.58, $p =0.023$) for dose 1×10^8. Other dose ranges not superior to placebo. S-EPs: improvement in, incomplete evacuation, passage of gas, training and bowel habit satisfaction in 1×10^8 group only. SGA 20% greater improvment than placebo ($p = 0.02$) 1×10^8 group only). No improvement in QOL in any group.

Author (Year published)	Participants (age Range)	Diagnostic criteria and sub-group	Length of treatment	Probiotic used	Outcome measures	Results of primary end point and other important results
Kim [244] (2005)	48 (21-75)	Rome II	8 weeks	*Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus plantarum,* and *Streptococcus salivarius ssp. thermophilus*	P-EP improvement in abdominal bloating score (VAS scores) S-EPs improvement in VAS scores for flatulence, abdominal pain and urgency. Proportion of weeks with satisfactory relief from bloating. (those patients who document at least 50% weeks of satisfactory relief declared responders.	P-EP: Satisfactory relief of bloating 46% vs. 33% in the probiotic and placebo groups (p = 0.27 NS). S-EPs: Vas score for flatulence (39.5 ± 2.6 (SD) vs. 29.7 ± 2.6 (SD)) probiotic vs. placebo (p = 0.01). No difference in all other symptom scores.
O'Mahony [245] (2005)	80 (18-73)	Rome II	8 weeks	*Lactobacillus salivarius ssp salivarius UCC4331* and *Bifidobacterium infantis 35624*	No priori P-EP specified. Symptoms of abdominal pain, bloating and bowel movement difficulty assessed (Likert scale and VAS score) and a composite score of all 3 calculated. IBS-QOL used to assess to quality of life.	Significant difference in abdominal pain (7.78 (SE 1.36) vs. 12.21 (SE 1.85)), ($p < 0.05$) bloating (10.17 (SE 1.67) vs. 14.39 (SE 2.18)) ($0.05 < P < 0.1$) in the *B. infantis* 35624 group over vs. placebo and composite scores (24.56 (SE 3.63) vs. 40.52 (SE 4.68)). ($p < 0.05$ after adjustment for multiple comparisons). No significant differences between the symptom or composite score for patients receiving *L. salivarius UCC4331* and placebo
Saggioro A [246] (2004)	70 (26-64)	Rome II	4 weeks	*Lactobacillus plantarum LP01 + Bifidocterium breve BR0* (group A) *Lactobacillus plantarum LP01 + Lactobacillus acidophilus LA02* (group B)	Overall pain score (4point Likert scale) Overall symptom severity score (4 point Likert scale).	Overall Pain: 45% group. A, 49% group B, 29.5% placebo Overall symptom score: 46% group A, 55.6% group B, 14.4% placebo (p values and statistical analysis not given).
Kim HJ [247] (2003)	25 (19-70)	Rome II IBS-D	8 weeks	*Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus plantarum,* and *Streptococcus salivarius ssp. thermophilus*	P-EP: satisfactory relief of symptoms. Responder defined as satisfactory relief. question (yes/no) of symptoms for 4 of 8 weeks. S-EPs: comparison of mean scores for individual IBS symptoms and bowel function (VAS scores).	P-EP: Responders 38% probiotic vs. 33% placebo (p = 1.00). S-EPs: No significant difference in any of the secondary end-points.

Author (Year published)	Participants (age Range)	Diagnostic criteria and sub-group	Length of treatment	Probiotic used	Outcome measures	Results of primary outcome and other important results
Niedzielin [248] (2001)	40 (27-63)	Manning	4 weeks	*Lactobacillus plantarum 299V*	P-EP: improvement in abdominal pain. S-EP: Improvement in overall symptoms score. Questionnaire of symptoms scores (rating scale) for abdominal pain, stool frequency and consistency, and flatulence.	
O'Sullivan [249] (2000)	24 (24-60)	Rome	20 weeks	*Lactobacillus casei GG*	P-EPs: Improvement in abdominal pain, bloating and bowel frequency S-EPs: Improvement in other symptom scores. Diary and questionnaires assessing abdominal pain, bloating, faecal urgency, diarrhoea, constipation, borborygmi, flatulence, belching, acid reflux, heartburn and nausea (Likert scales).	P-EPs: No significant difference between probiotic for abdominal pain, bloating or bowel frequency.
Nobaek [250] (2000)	60 (21-78)	Rome	4 weeks	*Lactobacillus plantarum DSM 9843*	No priori P-EP specified. Symptoms of abdominal pain, flatulence, defecation function (number and stool type).	Reduction in flatulence, 44% probiotic vs. 18% placebo reported a 50% reduction in flatulence ($p < 0.05$). Reduction in abdominal pain and improvement in bowl function in both groups but no significant difference between them.
Gade [251] (1989)	58 (16-60)	None	4 weeks	*Streptococcus faecium*	No priori P-EP specified. Clinician assessment of global symptom improvement. VAS scores for pain, bowel function, flatulence and meteorism, and boroborygmi	More patients 'improved' or 'significantly improved' in Clinician assessment in probiotic group than placebo, 81% vs. 41% ($p = 0.002$)

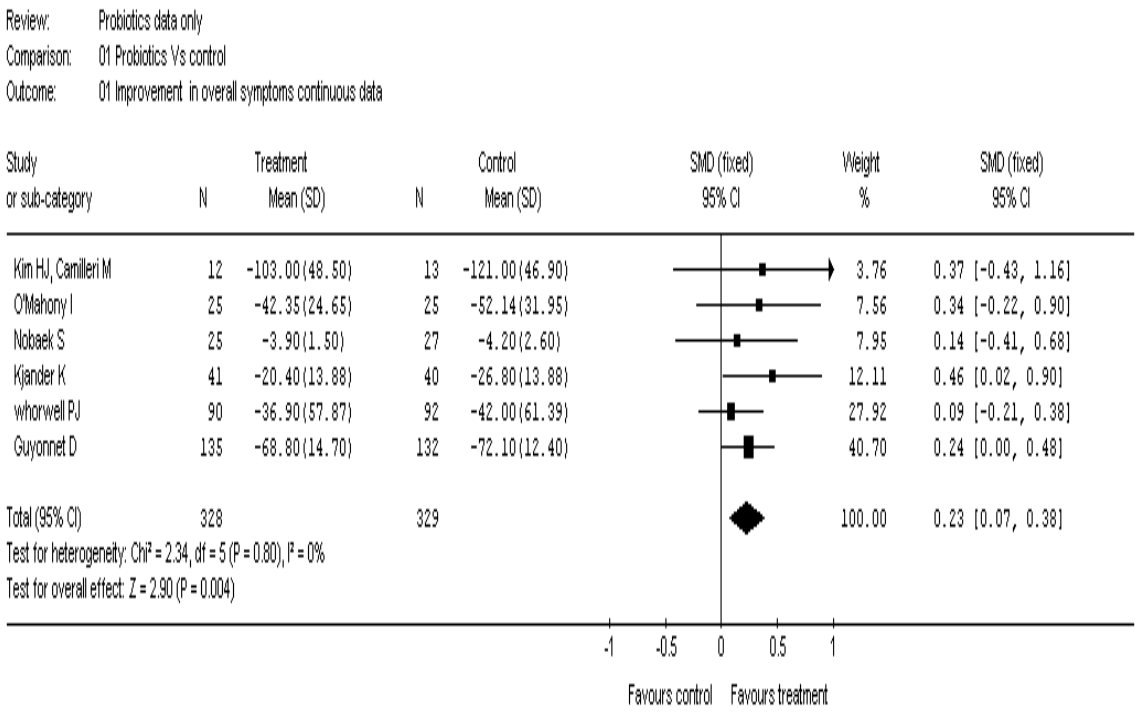
Summary of previous RCTs of different probiotics in the treatment of IBS. The studies included are those that are included in the previous meta-analyses of the subject. Gives number of patints included in study, age range, diagnostic criteria utilised in inclusion criteria, brief description of trial and major outcomes.(P-EP: Primary end point. S- EP secondary end-point)

Figure 1: Forest plot of improvement in overall symptoms (dichotomous data) in patients with IBS treated with probiotics compared to placebo.



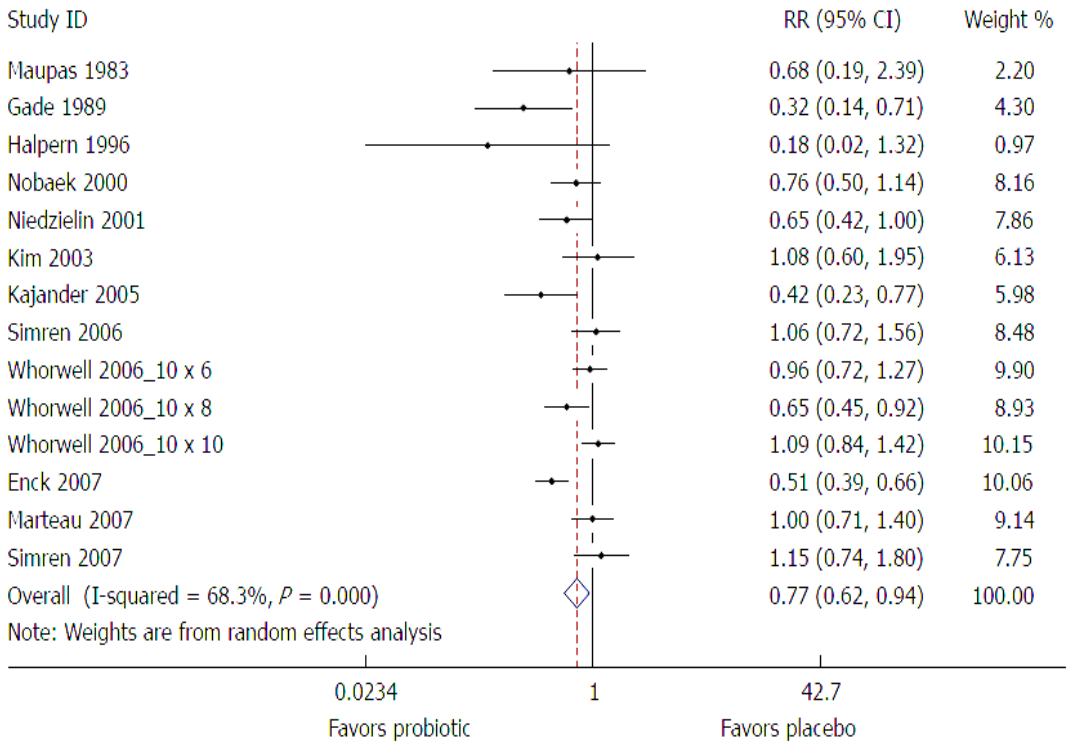
A forest plot from the meta-analysis by Hoveyda et al. comparing the dichotomous data (adequate relief of symptoms) from studies with suitable data for analysis. Compares placebo to probiotic in each cohort. Figure 1 is reproduced with permission from the original article by Hoveyda et al. [239]

Figure 2: Forest plot of improvement in overall symptoms (continuous data) in patients with IBS treated with probiotics compared to placebo.



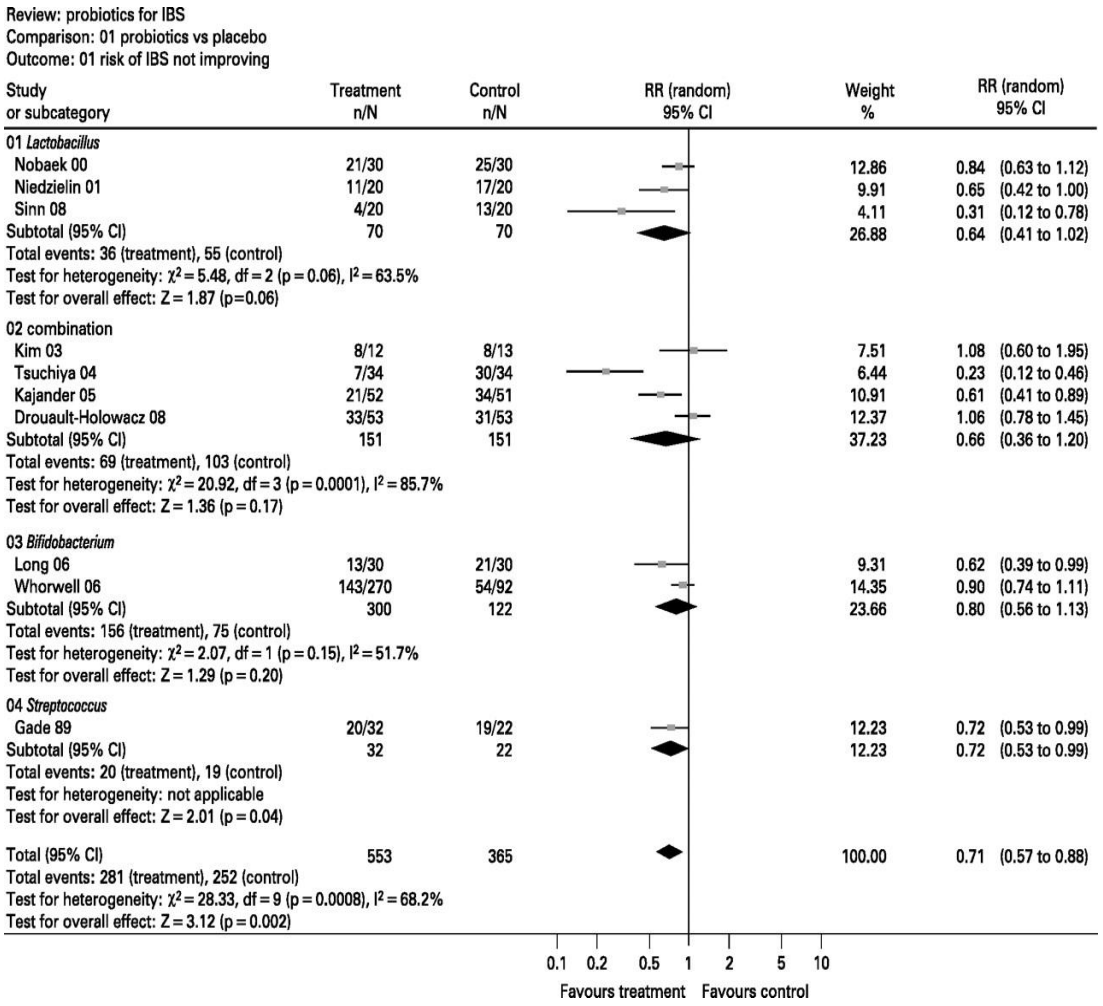
Forest plot of 6 trials included in the Hoveyda meta-analysis, Shows the improvement of overall symptom severity score, when reported using a continuous variable to report the outcome. Figures 2 is reproduced with permission from the original article by Hoveyda et al. [239]

Figure 3: Forest plot of randomised controlled trials of 14 treatment arms from 12 studies measuring the relative risk of IBS symptoms after probiotic treatment compared to placebo.



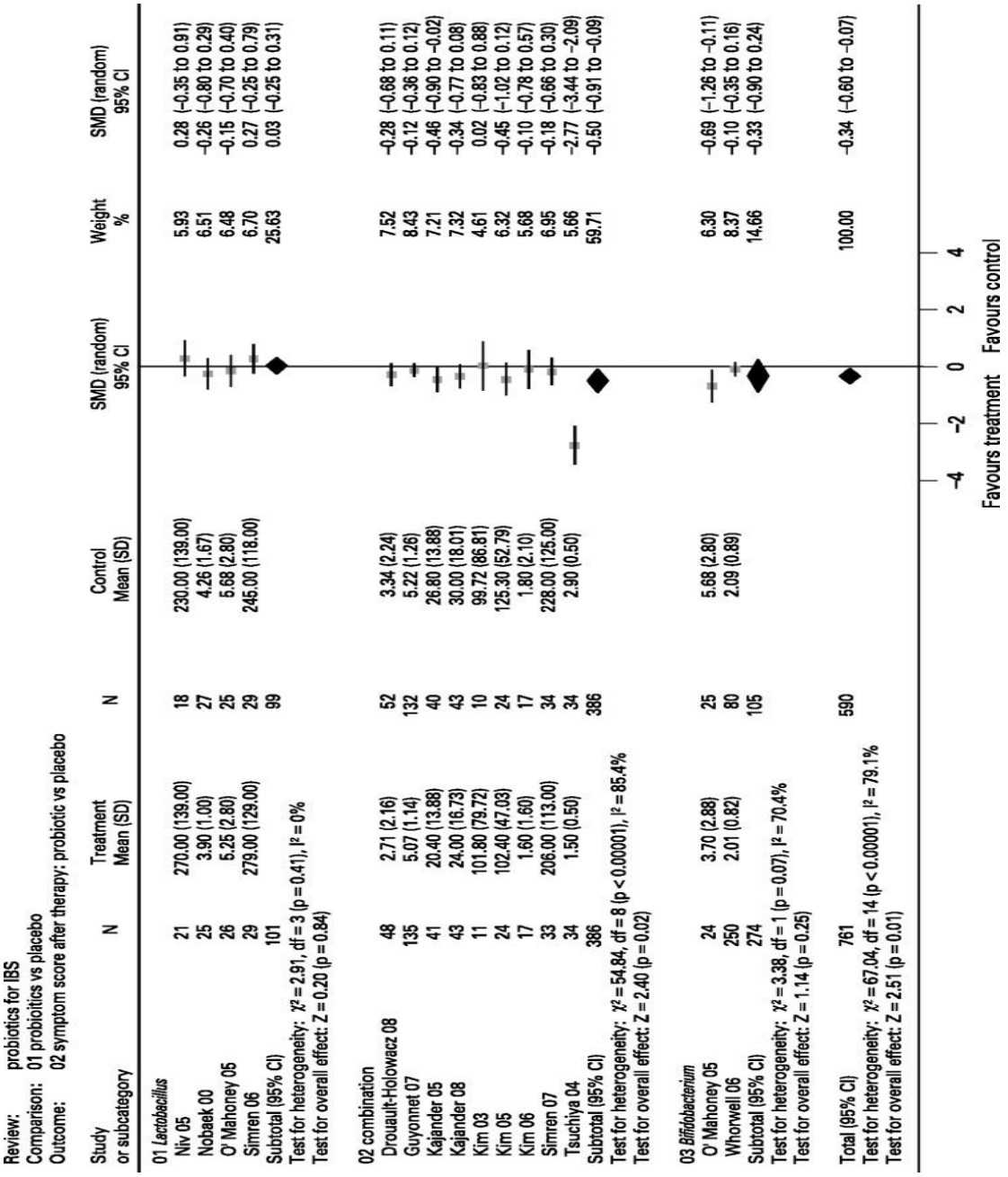
Forest plot of RCTs from 12 trials (14 treatment arms). Shows the relative risk (RR) of having IBS symptoms after treatment with a probiotic compared to placebo. Figure 3 is reproduced with permission from the original article by McFarland et al. [32]

Figure 4: Forest plot of trials comparing probiotics reporting a dichotomous outcome.



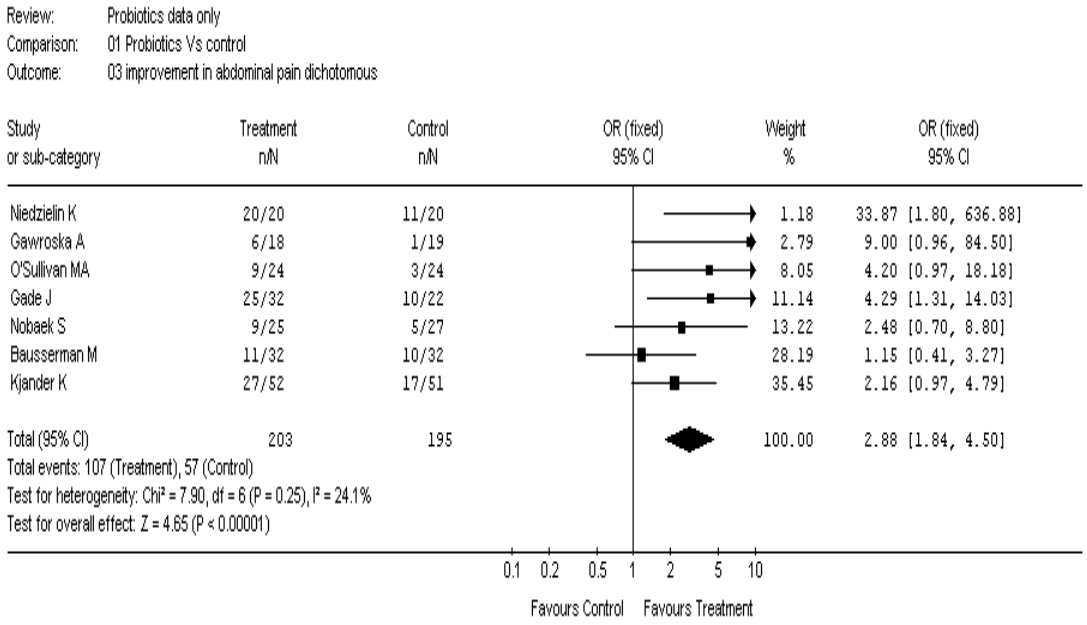
Forest plot from meta-analysis by Moayyedi et al. Shows the relative risk (RR) of persisting IBS symptoms after treatment with a probiotic compared to placebo using dichotomous data. The results are grouped according to probiotic species and/or if a combination product. Figure 4 is reproduced with permission from the original article by Moayyedi et al. [240]

Figure 5: Forest plot of trials comparing probiotics with placebo reporting a continuous outcome.



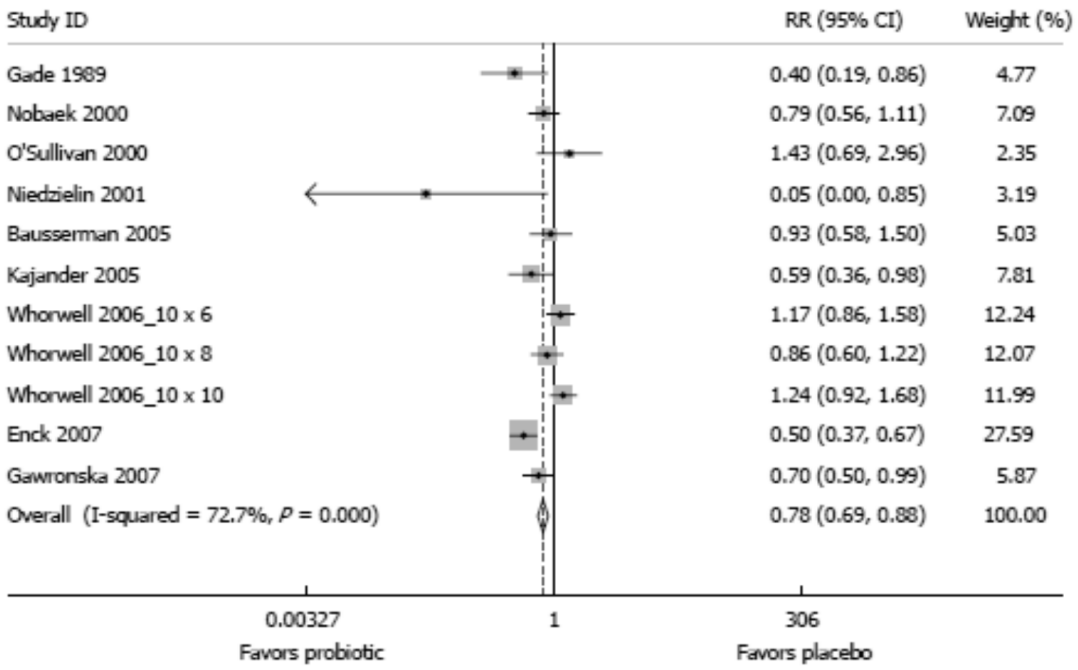
Forest plot from meta-analysis by Moayyedi et al. Shows the relative risk (RR) of persisting IBS symptoms after treatment with a probiotic compared to placebo using continuous data. The results are grouped according to probiotic species and/or if a combination product. Figure 5 is reproduced with permission from the original article by Moayyedi et al. [240]

Figure 6: Forest plot of improvement of abdominal pain (dichotomous data) in patients with IBS treated with probiotics compared to placebo.



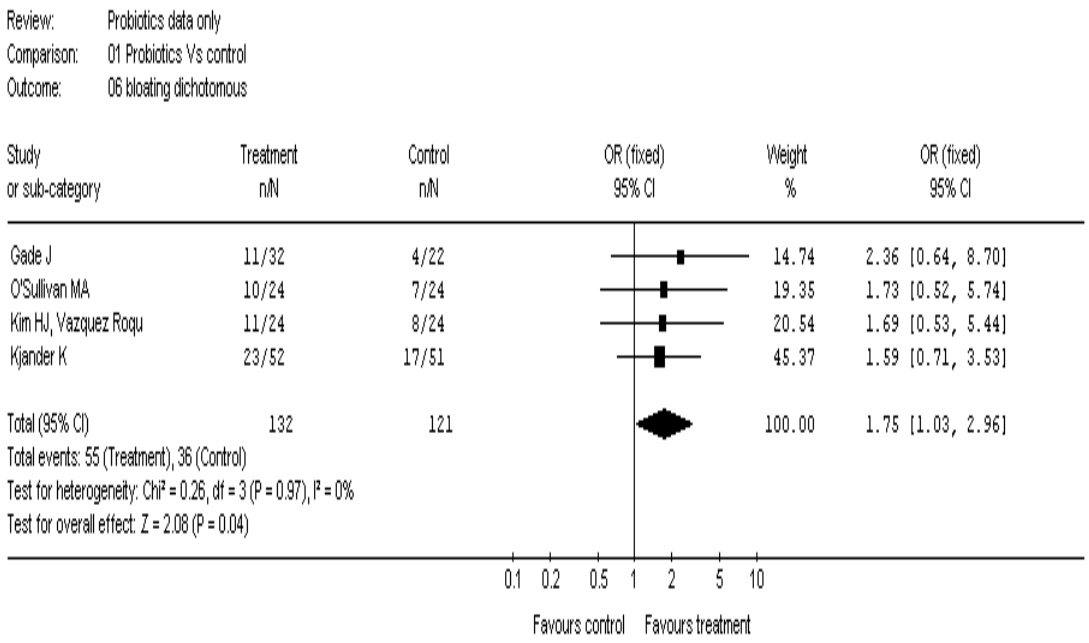
Forest plot from meta-analysis by Hoveyda et al. Shows the odds ratio (OR) of improvement in the symptom of abdominal pain in the different RCTs after treatment with a probiotic compared to placebo (dichotomous data). Figures 6 is reproduced with permission from the original article by Hoveyda et al. [239]

Figure 7: Forest plot of randomised controlled trials of 12 treatment arms from 10 studies measuring relative risk of abdominal pain after treatment with a probiotic compared to placebo.



Forest plot for meta-analysis by McFarland et al. showing the relative risk (RR) of abdominal pain in 10 RCTs (12 treatment arms) after treatment with probiotic compared to placebo. Figure 7 is reproduced with permission from the original article by McFarland et al. [32]

Figure 8: Forest plot of improvement of bloating (dichotomous data) in patients with IBS treated with probiotic compared to placebo.



Forest plot from meta-analysis by Hoveyda et al., showing the odds ratio (OR) of bloating from the included RCTs after treatment with probiotic compared to placebo (dichotomous data). Figures 8 is reproduced with permission from the original article by Hoveyda et al. [239]

Chapter 3:

Method

3.1 Ethics.

The study was conducted in accordance with the guidelines for Good Clinical Practice (CPMP ICH 135 95), the principals of the Declaration of Helsinki and with all relevant local and national guidelines including the archiving of records. The study protocol was reviewed by the Bromley National Research Ethics Committee (NRES) and given a favourable ethical opinion. Later amendments to the study protocol were reviewed by the Outer London NRES committee following the dissolution and amalgamation of the former committee into this new body. Further review was undertaken locally by the Research and Development Committee of Kings College Hospital who acted as Sponsors for the study. The study was registered on the ISRCTN register (International Standard Randomised Controlled Trial Number) (ISRCTN77512412).

All participants were provided with a full verbal description of the trial and a booklet containing detailed written information about the study protocol (appendix 1). After having an opportunity to ask questions, all participants were required to give informed written consent prior to enrolment in the study (appendix 2). Wherever possible data was stored in an anonymous format.

All computerised data was stored in an encrypted and password protected format in accordance with Data protection legislation. Original written data is stored securely by the sponsoring institution.

3.2 Study Design.

This study was a single centre, randomised, double blind, placebo controlled trial conducted in the gastroenterology outpatient department of Kings College Hospital, London. The study took place between October 2008 and September 2011. A total of 186 participants were recruited and randomised. Study participants received either active treatment with the study probiotic suspension or placebo. The placebo was an inert liquid (water) containing natural citrus flavourings only. Participants were allocated to receive active treatment or placebo randomly, (section 4.2.3) in a 2:1 ratio giving 124 patients on active treatment and 62 patients on placebo. This study was designed as a 'Phase II' study, to examine both the efficacy and safety of the probiotic preparation in the target population. The probiotic suspension has been commercially available for human consumption for several years, however, the safety profile for the preparation has not been studied in a controlled format. Therefore, within the study design, it was decided to include a larger number of patients taking probiotic than placebo in order to potentially yield more information about its safety profile and any unwanted effects.

The study period was for a total of seventeen(sixteen plus one) weeks, which included one week of pre-assessment, twelve weeks of treatment and

four weeks of follow up. The full timetable of visits and interactions with participants is given in Table 5.

3.2.1 Participant Selection and Screening.

Participants for the study were selected in two ways: Direct from tertiary care at Kings College hospital and from Primary Care from a number of local GP clinics. Participants with IBS, under the care of a gastroenterologist at Kings College Hospital, or patients newly referred directly to the gastroenterology service with a suspected diagnosis of IBS were informed about the study and given an information sheet during their routine outpatient appointment. Patients who then showed an interest in participation were offered a separate initial assessment appointment with a member of the research team.

Potential participants from Primary Care were identified from the computer databases of two local primary care facilities (General Practice (GP)) that consented to participate in the study. Using these databases, patients with an established diagnosis (made by their GP) who had consulted with the GP within the preceding 12 months regarding their IBS symptoms, or had received a prescription for mebeverine during the same time period, were identified. Potential participants were then sent an open information letter from the GP , the study information booklet and a separate invitation letter from the research

group inviting the GP patients to take part in the research study. Patients who subsequently contacted the research team, were then offered an initial assessment appointment with a member of the research team in the outpatient department at Kings College Hospital.

Those respondents who consented, attended an initial screening outpatient assessment (visit 1) during which demographic data was collected, a detailed clinical history was obtained and participants underwent a full clinical review. Participants who had not previously been diagnosed with IBS by an experienced gastroenterologist at Kings College hospital, underwent standard screening investigations to establish the diagnosis. Patients who were currently under the care of an experienced gastroenterologist at Kings College Hospital, had these investigations repeated if they had not been performed in the one month prior to screening. All patients underwent faecal calprotectin and those with diarrhoea also underwent a small bowel permeability study (SBPS) within the two weeks prior to study entry. Small bowel permeability was assessed using the method describe by Menzies et al. that analyses urinary excretion of orally administered sugars (D-xylose, 3-O-D-glucose lactulose and Rhamnose). [252]

Where the history, clinical examination or investigations indicated a diagnosis other than IBS, the investigator initiated appropriate investigations and the participant's involvement in the study was suspended until these were

completed. If these further investigations resulted in either a confirmed, or 'strongly' suspected alternative diagnosis, then participants were reassessed as to their suitability to take part in the study and excluded if they no longer met the entry criteria. In cases where there was any doubt about the diagnosis, the final decision as to inclusion to the study was made by the Principle Investigator. Any patients in which an alternative diagnosis was reached as a result of the screening investigations, were referred for investigations and treatment with an appropriate clinician.

3.2.2 Diagnostic Criteria for IBS.

Currently there are no specific investigations or panel of tests available to positively confirm a diagnosis of IBS. A diagnosis of IBS is therefore based on the detailed assessment of an individual's history of symptoms and clinical findings by an experienced physician, as to whether they meet a set of established symptom criteria in the absence of an alternative explanation for the symptoms. For the purpose of this study, participants were only considered to have a confirmed diagnosis of IBS if they met the Rome III criteria.[5] The compliance of a patient's symptoms with The Rome III criteria were assessed using a simple questionnaire adapted from the Rome III diagnostic criteria (Table 6). Once the diagnosis of IBS was confirmed it was then necessary to establish whether the patient was currently symptomatic. For the purpose of this study patients were considered to have symptomatic IBS if they had an IBS

symptom severity score (IBS-SSS) of ≥150. The level of ≥150 was chosen based on information from the validation studies of the IBS-SSS; in which normal subjects or those with quiescent symptoms are considered as having an IBS-SSS of ≤75 and that a meaningful difference in the IBS-SSS score would be a change of ≥50 points. A cut off point of ≥150 was therefore chosen for inclusion in the study, to reasonably allow demonstration of a meaningful change in the IBS-SSS in response to the study treatment.

3.2.3 Inclusion and Exclusion Criteria.

The inclusion and exclusion criteria were chosen in order to ensure that only patients with a diagnosis of IBS were included in the study, and to minimise the effects of potential confounding of co-morbid conditions on the outcome from the study. All sub groups of IBS were included within the study.

Inclusion Criteria:

- Age 18-65 years at screening visit.
- Male or Female.
- Diagnosis of IBS made by an experienced Gastroenterologist and Rome III diagnostic criteria met. (Rome III assessment performed by study physician).

- Commencement of symptoms at least six months prior to screening visit.
- Current active disease (defined as IBS-SSS score of ≥150)
- Willingness and ability to give informed written consent
- Willingness and ability to complete questionnaires during the study.
- Willingness to undergo pre- and post-study investigations as per the study protocol.

Exclusion Criteria.

- Age <16 or >65 at screening.
- History of confirmed diagnosis (histological and/or endoscopic) of other gastro-intestinal diseases:
    - Crohn's disease.
    - Ulcerative colitis.
    - Microscopic/Collagenous colitis.
    - Coeliac disease.
    - Small bowel bacterial overgrowth.
    - Diverticulitis/Diverticulosis.

- Gall stone disease (permitted if undergone cholecystectomy >1 year previously and confirmation of absence of residual common bile duct stones).

- Gastroparesis.

- Previous or current significant psychiatric co-morbidity.

  (Mild anxiety and depressive symptoms were accepted at the discretion of the screening physician. Patients currently under investigation; suffering from a recent or on-going depressive episode, requiring a change in treatment during the preceding three months or treatment other than low dose anti-depressants were excluded).

- Anti-depressant usage: low dose anti-depressants prescribed as part of IBS treatment were permitted, e.g. citalopram 10-20mg daily, amitriptyline 10-20mg daily.

- Current alcohol misuse or dependency.*

- Current drug misuse or dependency.*

  (* Previous misuse or dependency permitted at discretion of screening physician if >five years prior).

- Pregnancy.

- Probiotics:

- Previous use of a probiotic preparation within one month of screening.
- Previous adverse reaction to probiotic preparation.

(Patients were instructed to avoid all other probiotic preparations during the study period).

- IBS medications:

(Patients already taking IBS medications including anti-spasmodics, peppermint preparations or low dose antidepressants, on regular or as required bases were permitted to continue using them in the same manner during the study. Increased dosages or new medications were not permitted during the study.

- Dietary restrictions and elimination diets:

(Patients who were already on an established wheat and/or dairy elimination diet, or had self-imposed dietary restrictions for a period of not less than three months prior to the start of the study were permitted. Patients were instructed not to change diets during the study period. Patients were asked to refrain from major dietary changes during the study period). Patients being treated using the FODMAP exclusion diet were excluded from the study.

- Antibiotics:

The use of antibiotics was not restricted during the study period. Antibiotic usage during the study period was recorded as a ‘study variance’ during follow up visits.

3.2.4 Study medication.

During the study patients received either placebo (or probiotic in a dose of 1ml per kg each day during the treatment phase. Patients were requested to take the medication each morning on an empty stomach and eat or drink nothing other than clear fluids for at least 10 minutes after taking the probiotic/placebo. If patients forgot the morning dose they were instructed to take the medication later in the day when remembered, with a period of no food for at least two hours prior to consumption.

3.2.5 Patient visits and assessment.

All clinical assessments, investigations and monitoring visits during the study were performed by the lead physician. At each assessment point during the ‘active treatment’ phase, all study participants had a separate meeting with

a non-clinical research assistant to receive their next supply of study probiotic or placebo. The research assistant was not blinded and took no other part in any other clinical aspects of the study.

At ‘visit one’ the lead physician provided full verbal and written information regarding the study; then with consent, undertook a full review of the participants medical history and conducted a full physical examination. If the history was clearly not consistent with a diagnosis of IBS, appropriate alternative investigations and intervention were undertaken and the screening process stopped. These participants took no further part in the study. Participants whose history and physical examination was consistent with a likely diagnosis of IBS who had not been previously investigated had the following laboratory investigations arranged:

- Full blood count. (FBC)
- Erythrocyte sedimentation rate. (ESR)
- C-reactive protein. (CRP)
- Renal profile. (urea, creatinine, potassium and sodium).
- Liver function tests (Albumin, globulin, bilirubin, alanine transaminase (ALT), gamma-glutamyl transpeptidase (G-GT) and alkaline phosphatase (ALP)).

- Coeliac serology (anti-tissue transglutaminase (tTG), anti-endomysial, anti-gliadin antibodies and serum IgA level.
- Small bowel permeability study. (if IBS-D or IBS-M)
- Faecal calprotectin.

If participants already had an established diagnosis of IBS made by an experienced gastroenterologist and had undergone previous laboratory investigations, they were repeated as per the following protocol:

- FBC, CRP, ESR, faecal calprotectin, small bowel permeability study – if not done within four weeks of entry into the study.
- Coeliac serology – repeated if not done within six months.

Participants who were thought to be likely candidates to take part in the study and gave verbal consent, were asked to complete the IBS-SSS questionnaire to assess symptom severity and thus eligibility for inclusion in the study.

At ‘visit two’, the laboratory investigations were reviewed and if participants had abnormal laboratory investigations, or the lead investigator

suspected an alternative diagnosis, then further investigations were arranged and the participant was suspended from the trial assessment.. The participants requiring further investigations had a repeat ‘visit two’ arranged to review these and reassess study eligibility. Participants were excluded from the trial if further investigations confirmed or suggested an alternative diagnosis to IBS was likely. In unclear cases the final decision regarding diagnosis was made by the lead investigator and confirmed by the principal investigator prior to inclusion in the study.

Patients who had normal laboratory investigations, an IBS score of ≥150 and met the Rome III criteria as assessed by the study questionnaire, (Table 6) were then invited to participate in the study. Written consent was completed at ‘visit two’ and the participant enrolled. Each participant was assigned the next available sequential trial identification number and completed the appropriate questionnaires as required by the study protocol. (Table 5). All participants were given a study identity form that included the trial identification number and emergency contact details for the trial team.

During the study, participants were reviewed every four weeks by the lead investigator. At each visit, participants were asked about compliance with the study treatment, any unwanted side effects or adverse events and any variances from the study protocol; including any other medications used during this period. Compliance with study medication was assessed at each of the four

weekly visits during the treatment phase of the study. Participants were asked specifically if they had taken each daily dose, missed one or less doses per week, missed one-three doses per week or missed greater than three doses per week, at each visit.

Study questionnaires were completed as per the study protocol. Participants who were experiencing significant side effects or adverse events or withdrew consent, were withdrawn from the study and the reason recorded. Those participants who failed to attend a follow up appointment were contacted and offered a further appointment one week later. Participants who did not attend two consecutive follow up appointments or could not be contacted were withdrawn from the trial. Every effort was made to contact participants who failed to attend.

At ‘visit five’, week twelve of the study, participants were asked to stop taking the study probiotic/placebo and underwent the end of study investigations. (FBC, Renal profile, liver function tests, CRP, ESR, small bowel permeability (if IBS-D or IBS-M) and faecal calprotectin) Study compliance, variances, side effects and adverse incidents were recorded.

A four week follow up period where patients took no further medication then followed before the final review (visit six). Final information was collected

as per the protocol and the participants involvement in the study concluded. A further appointment was also made for each participant to meet the Principal Investigator after ‘visit six’. At this meeting the participant was ‘unblinded’ and as required further treatment and follow up was discussed. The Principle Investigator remained ‘blinded’ to the patients study identification number but was ‘unblinded’ to whether they received probiotic or placebo. This additional step in the blinding protocol was taken to ensure that informing the patient of their allocation to placebo or probiotic, did not comprise the ‘blinding’ of the Principle Investigator.

3.2.6 Randomisation and Blinding.

The study protocol included a total of 186 patients with twice as many in the probiotic group compared to the placebo group. Participants were therefore randomised into three groups of equal size. Two active treatment groups and one placebo group giving an overall ratio of active treatment: placebo of 2:1. The decision to use three equal sized groups was to ensure investigator blinding. The groups were compiled using a simple non-stratified two stage computer randomisation protocol, utilising the Mersenne twister algorithm, a pseudo-random number generator to randomise participants equally into each of the three groups. The full randomisation for all 186 patients was completed prior to the start of recruitment. At the screening visit each participant was

assigned the next sequential trial number by the Lead Investigator. The randomisation protocol is illustrated in Appendix 3.

Two copies of the randomisation sequence were kept during the study. One held by the research assistant, (non-clinical) who was responsible for allocation of the probiotic/placebo during the study, and a second 'emergency' copy that was held in a sealed security file during the study; to be accessed in the unlikely event that there was a need to 'unblind' for a study participant in case of a medical emergency. The Lead Investigator remained blinded throughout the study and was responsible for all clinical contact with participants during the study period. At the end of their involvement in the study each participant was reviewed and 'unblinded' by the Principal Investigator, who also arranged and carried out all follow-up care required. The Lead Investigator had no further clinical contact with participants after completion of the study to ensure maintenance of blinding.

The placebo and probiotic were provided and distributed in identical bottles in sealed, identical cardboard boxes. The content of the boxes was identified by an eight digit numeric code assigned by the manufacturer and known to the research assistant only. The probiotic and placebo were, as far as possible, similar in taste, colour and consistency. The placebo was an inert liquid (water) with added natural flavourings of citrus.

All data collected during the study was entered in to a secure, encrypted database in a pseudo-anonymised form. All data was collated into the three treatment groups and the database locked prior to ‘unblinding’

3.3 Laboratory investigations.

Haematological and biochemical tests were carried out using Adviva 1200 and 2400 analyzers respectively, (Siemens, Frimley, UK) intestinal permeability assessment (differential urinary excretion of lactulose / L-rhamnose) using mass spectrometry for marker analyses and faecal calprotectin (EK-Cal Calprotectin kit, Buhlmann, Switzerland). All laboratory investigations and measurements were carried out by the Department of Clinical Biochemistry, King’s College Hospital, as previously described. [253, 254]

3.4 Outcome measures

3.4.1 Questionnaires.

The main outcome measurements of the study were a change in the symptom severity of the participants in response to treatment with either the probiotic or placebo. At the time the study commenced there was no universally accepted standard to assess response to treatment in IBS clinical trials. For the purposes of this study, it was felt that the use of independently developed and validated symptom severity and QOL questionnaires should be utilised, to maximise the relevance and application of the study and to facilitate comparison with other IBS clinical trials.

Symptom severity was assessed using the IBS-SSS (IBS symptom severity score) developed by Francis et al. [255] The IBS-SSS utilises five simple questions to assess the severity of the principle symptoms of IBS namely: Abdominal pain, bloating, bowel habit dysfunction and satisfaction with bowel habit. Each of the four symptom domains are assessed using a 100mm visual analogue scale (vas). Pain is further assessed for duration; by the number of days a patient has experienced pain over the preceding ten days, which when multiplied by ten gives a further score out of 100. This results is an increased weighting within the score for abdominal pain; as it includes scores for both duration and severity, which is line with the consensus opinion that pain is the single most important symptom for patients with IBS. The IBS-SSS questionnaire thus yields an overall score range of 0 to 500. The original authors have validated the score for sensitivity to change and reproducibility, and from their study concluded that a score of ≤75 indicated no symptom activity and was comparable to scores obtained from the control group. A score

of >75 to ≤175 was consistent with mild symptoms, >175 to <300 with moderate symptoms and ≥300 with severe symptoms. They also concluded that a change in the IBS-SSS score of ≥50, represented a meaningful change in symptoms with a sensitivity and specificity of 76% and 68% respectively. [255] The IBS-SSS questionnaire is shown in Appendix 4.

The IBS-QOL score is a specific QOL score for IBS sufferers utilising a 34 item questionnaire. It was developed by Patrick et al. and based on a combination of outcomes from conceptual models of generic and gastro-intestinal specific health related quality of life questionnaires, the bothersomeness of IBS symptoms as reported by organised focus groups, and specific qualitative interviews with patients. [256] The IBS-QOL score evaluates eight individual domains (dysphoria, interference with activity, body image, health worry, food avoidance, social interaction, and sexual relationships) and gives an overall QOL score out of 100. For the purpose of this study the scores for individual responses to each item were scored in a negative manner, (e.g. a response of ‘not at all’ would score four, whereas ‘extremely’ would score zero) This is not the conventional way of scoring this questionnaire and results in a higher score indicating a better quality of life, however it does not alter the quantitative values of the individual scores or scale. The IBS-QOL score was validated by the authors for internal consistency and reproducibility, as well as comparability with symptom severity and other established generic and gastro-intestinal specific health related quality of life instruments. The IBS-QOL questionnaire is shown in Appendix 5.

The IBS-SSS questionnaire was completed at the beginning and end of the pre-study assessment week (week -1 and week 0). The IBS-QOL reports on quality of life over the preceding four weeks and was therefore only completed at week 0 to yield baseline data. The questionnaires were completed at each time point according to the study protocol (Table 5)

3.42 Primary and Secondary Outcome Measures.

In the original study protocol the primary outcome measure was a change in the IBS-QOL score. However, a further review of other similar clinical studies of new treatments for IBS highlighted the fact that a change in QOL was difficult to demonstrate, even when a significant improvement in individual and global symptoms was observed. Moreover, it is also demonstrated that there may be a lack of congruity between improvement in symptoms and changes in QOL measurement. The original authors of the IBS-QOL have validated their questionnaire to demonstrate construct validity, internal consistency reliability and reproducibility. However, this has not been further validated externally.

After consideration of this information the IBS-SSS was used as the main outcome measure, and difference in the change in IBS-SSS from baseline to week twelve between the probiotic and placebo group was considered the

primary end point. The pre-study power calculation was therefore calculated on achieving a difference in the change in the IBS-SSS between the two groups. The IBS-QOL was still included but as a secondary end-point.

The secondary end-points for the study were; change in the IBS-QOL at 12 weeks; change in either the IBS-SSS or IBS-QOL at other time points (4, 8, and 16 weeks); and a change in the IBS-SSS individual composite scores for pain, bowel habit, bloating and QOL.

3.5 Study probiotic and placebo preparation.

This study was desired to assess the safety and tolerability efficacy of a multi-strain liquid probiotic. The probiotic used is a liquid preparation prepared for the study by the Symprove Ltd., The Sands Business Centre, Farnham, Surrey. GU10 1PX. The preparation method utilises a fermentation process with germinated barley as a substrate/growing media to culture the specific probiotic strains. The product contains the following four bacterial strains:

- *Lactobacillus plantarum NCIMB 30173*
- *Lactobacillus rhamnosus NCIMB 30174*

- *Lactobacillus acidophilus NCIMB 30175*
- *Enterococcus faecium/durans NCIMB 30176*

A 50ml dose of the probiotic contains approximately 10^9 colony forming units (CFUs) i.e. individual bacteria that are live and capable of replication. The probiotic is maintained in a liquid form, with live activated bacteria or CFUs in a suspension of an extract of the fermentation liquid to maintain the bacterial viability. The probiotic is gluten and dairy free. The probiotic preparation has been shown, in an independent study commissioned by the manufacturers, to remain stable and biologically active (continues to replicate) after exposure to a solution of acidity comparable to that of the 'resting' human stomach, (pH 3) for thirty minutes.

The placebo consisted of sterile water with the following additions (of water 130g of ascorbic acid and 3g of beta carotene per 25 l water). The additions were included to give the placebo a taste and appearance similar to the probiotic preparation. The final placebo contained (0.26g ascorbic acid and 0.006g beta carotene per 50ml measure). Quality assurance for the probiotic and placebo where carried via and independent QA laboratory.

3.6 Statistical analysis.

Statistical analysis was conducted using STATA 12.1 and SPSS 21 statistical software. The statistical analysis for the primary end point and related analysis was performed by and/or under the supervision of Dr Salma Ayis, lecturer in Statistics, Kings College London.

The original power calculation to discern the total number of patients for inclusion in the study was derived from the assumption that 40% of patients in the placebo arm would show an improvement in the IBS-SSS, whilst the probiotic group would show a similar reduction in IBS-SSS score in 65% of patients, detected with a power of 90%. A change in the base-line score of 50 points was considered as the minimal clinically important difference (MCID)

The statistical analysis was performed using both a per-protocol (PP) and intention to treat (ITT) approach. All patients that received study medication were included in the ITT population and all patients that completed the twelve weeks active treatment phase were included in the PP population. For the ITT analysis. the missing data from patients who were lost to follow up, withdrew before completion of the study or data lost due to administrative error was generated using the last observation carried forward principle. (LOCF)

[257] A comparison of the PP and ITT (LOCF) analyses was used as a basic sensitivity analysis when comparing the data.

Frequency tables and cross-tabulations were derived to explore any differences or associations between different variables and/or baseline characteristics. Modelling with ordinary linear regression was used to further analyse the primary end point. Residual plots were used to test the regression models.

The primary and secondary efficacy measures were analysed for the per-protocol (PP) and an ITT basis using independent sample t-test. Where repeated analyses were conducted on multiple secondary endpoints within the same data, one-way ANOVA was used. Adjustment for potential confounding baseline variables was conducted using analysis of covariance (ANCOVA). Pearson-Chi-squared analysis was used to compare the proportions of subjects achieving mild or no symptoms within the two groups in a post-hoc analysis. A p value at or below 0.05 was considered a significant result.

Table 5 : Visit and Investigation and Questionnaire Protocol.

Week No.	Visit	Actions/Interventions	Study Treatment	Questionnaires
-1	Visit 1 Screening	History & examination Laboratory tests		IBS-SSS
0	Visit 2* Consent, Randomisation and enrolment	Test results Consent/randomisation (*further tests)	Start Probiotic/placebo	IBS-SSS IBS-QOL PSQI
4	Visit 3 Assessment and monitoring	Monitoring ** Collection of questionnaires		IBS-SSS IBS-QOL PSQI
8	Visit 4 Assessment and monitoring	Monitoring ** Collection of questionnaires		IBS-SSS IBS-QOL PSQI
12	Visit 5 Assessment and monitoring	Monitoring ** Collection of questionnaires End of study laboratory tests+	Stop Probiotic/placebo	IBS-SSS IBS-QOL PSQI
16	Visit 6 Final assessment and monitoring Visit 6(b) Patient unblinding ++			IBS-SSS IBS-QOL PSQI

Schedule of visits for study, includes description of individual elements and purpose of each visit.

*At visit 2 further investigations will be initiated if clinically indicated and a further ‘visit 2’ review arranged

** Monitoring visits – include recording of variances, compliance, side effects, adverse events and serious adverse events.
+ Faecal calprotectin, CRP, ESR, FBC, renal/liver/bone profiles and small bowel permeability study.
++ Visit 6(b): Study participants will be reviewed by the Principal Investigator to be unblinded (all other clinical interactions will be performed by the lead investigator who will remain blinded)

Table 6: Rome III Criteria Questionnaire.

		Question	Answer
1		Do you currently experience recurrent abdominal pain or discomfort* (*if yes then go to question 2)*	YES/NO
2		Over the last 3 months at this pain/discomfort occurred for at least 3 days per month	YES/NO
3	a	Does the abdominal pain/discomfort improve with defecation (passage of stool/faeces)	YES/NO
	b	Is the onset of this abdominal pain/discomfort associated with a change in the form of stool (appearance e.g. – change from hard stool to runny or soft stool)	YES/NO
	c	Is the onset of this abdominal pain associated with a change in the frequency of stool (eg. having a bowel movement, more or less often than usual for you)	YES/NO
4		Did your current symptoms original start at least 6 months ago.	YES/NO
5		Over the last one month have these symptoms occurred on at least 2 days each week **	YES/NO

Description of individual questions used for study inclusion to ensure participants diagnosis of IBS is concordant with the Rome III criteria.

* Discomfort – is an uncomfortable sensation that is not described as pain

** Question 5 – this is not a requirement of the Rome III criteria but is suggested as additional measure by the Rome III consensus working group when considering patients for entry into clinical trials.

The Rome III diagnostic criteria for IBS are met if the answers to questions 1, 2, 4,and at least 2 components of question 3 are yes.

Chapter 4:

Results.

4.1 Cohort demographics.

4.1.1 Recruitment.

Study recruitment commenced in October 2008 and was completed by July 2011. A total of 392 patients were screened for eligibility to take part in the study. Of these 191 were recruited and took part in the study. A total of 201 patients that were screened were not included in the study. The reasons for exclusion from the study are; 52 patients did not give consent to take part; 39 did not attend their next appointment and were not randomised; and 41 patients had a diagnosis of IBS but were not symptomatic as defined by the study protocol (IBS-SSS of ≥150). 50 patients with a previous diagnosis of IBS were confirmed to have a diagnosis other than IBS (simple constipation, other functional gastro-intestinal disorders (not IBS) diverticular disease, inflammatory bowel disease, gynaecological disorders, drug related diarrhoea, coeliac disease, HIV related diarrhoea, post infective gastroenteritis diarrhoea (<4 weeks duration) and small intestinal bacterial overgrowth). Seven of the remaining patients were excluded as a result of significant psychiatric co-morbidity and 12 were excluded for not meeting one or more of the other inclusion criteria (pregnancy, age >65, long-term antibiotics, severe liver disease, on anti-cancer therapy, recent cardiac arrest, enrolled in separate clinical trial).

A total of 191 patients were recruited to the study. Five patients were recruited and allocated a trial participant number at ‘visit 1’. However, they did not attend their next appointment (visit 2) at the end of the screening period to receive the first allocation of study medication. It was decided that as these patients did not receive any probiotic or placebo and investigator blinding would be unaffected it would be reasonable to reallocate these trial identification numbers. The CONSORT flow chart of study recruitment and withdrawal is represented in figure 10.

4.1.2 Baseline Demographics and Investigations.

4.1.2.1 Age and Gender.

The overall age range of participants in the study was 18-65 with a mean age of 38.3 years (SD ± 10.6). In the probiotic group the mean age was 39.1 (SD ± 10.5) and placebo group 36.8 (SD ± 10.7) and there was no significant difference between the groups ($p = 0.162$). The mean age did not very according to gender with mean ages of 39.8 (SD ± 10.5) and 37.7 (SD ± 10.7) in males and females respectively ($p = 0.21$).

Significantly more females (129) than males (57) were recruited to the study. The female to male ratio within the study was 2.26:1 which reflects the known female predominance of IBS prevalence in the UK. There was no significant difference in the ratio of males to females between the active (2.1:1) and placebo (2.6:1) treatment groups (p = 0.50), (*Pearson Chi-square method*). (table 7)

4.1.2.3 Disease sub-group and duration.

The allocation and randomisation of patients to the study groups was not stratified by disease duration of sub-group. Disease duration has been sub-divided into 3 categories, (< 1 year, 1 to 5 years and greater than 5 years). There are no significant differences between the proportions of each category in the active and placebo groups (table 7). Similarly there are no significant differences between the proportions of disease sub-type, based on stool characteristics, between the groups (table 7). It is of note that 136 (73.1%) of the patients recruited to the study had either IBS-M or IBS-D, with only 40 patients having IBS-C and 10 patients IBS (unclassified). The low proportion of patients with IBS-C and IBS means than meaningful sub-group analysis of these sub-groups is not feasible.

4.1.2.4. Medications and miscellaneous characteristics.

Details of the number of patients taking individual medications is given in table 8 (some patients were taking more than one medication). The use of medication between the group did not differ significantly other than in the use of SSRIs. Whilst low dose SSRI were permitted in the inclusion/exclusion criteria it is noted that there are substantial more patients (13 vs. 2) in the probiotic group compared to placebo. Table 8 contains other additional information on baseline demographic information but no significant differences occurred between the treatment and placebo groups.

4.2 Baseline IBS-SSS

The IBS-SSS was measured at week -1 and week 0 in all subjects. The reason for measuring it at two time point was to give an indication of the degree of variability of this measure at study entry. There was no significant difference between the mean IBS-SSS score at week -1 (307.5, sd ± 71.7) or week 0 (300.9, sd ± 78.5) score for the whole cohort (p = 0.067, CI: -0.460 – 13.685) or in the active treatment group; week -1 (305.3 sd ± 69.1) and week 0 (302.1, sd ± 74.8)(p = 0.479, CI: -5.599 – 11.860). In the placebo group there was a significant difference between the IBS-SSS scores between week -1 (312.5, sd ± 76.9) and week 0 (298.5, sd 85.9)(p = 0.031, CI: 1.288 – 25.746). There were

no significant differences in the week -1 or week 0 scores between the probiotic and placebo group (table 9). The apparent difference in the placebo group between week -1 and week 0 is suggestive of a small improvement in symptom severity prior to the commencement of the treatment phase of the study. In light of the observed difference in the scores in the placebo group an average score of the week -1 and week 0 scores was calculated. The mean 'average IBS-SSS' score for the whole cohort was 304.4 (SD ± 71.4). The mean in the probiotic group was 303.6 (SD ± 68.0) and the placebo group 306.0 (SD ± 78.2) (table 9).

Subsequent analysis shows that there was no difference between the mean 'average IBS-SSS score at baseline in the probiotic group (303.6, SD ± 68.0) and the placebo group (306.0, SD ± 78.2)(p = 0.832, CI: - 24.940 – 20.090). The calculated 'average baseline IBS-SSS was therefore used as the reference point for statistical analysis. The overall distribution of the IBS-SSS at baseline conforms to the normal distribution and is similar across both the active and placebo group. Figures 11 – 13 show the distribution of the IBS-SSS score at baseline.

As discussed, it was noted that there was an excess number of patients in the probiotic group taking low dose SSRIs or amitriptyline at the start of the study. A further analysis of the IBS-SSS at baseline excluding those patients taking this medication showed no significant difference between the groups with

the probiotic group having a mean baseline IBS-SSS of 303.5 and the placebo group 304.9. (CI: -25.12 – 22.23)(p = 0.90).

A model using ordinary linear regression was performed to explore whether the IBS-SSS score at baseline was affected by age, gender, duration of disease or disease subtype. The results are shown in table 10, none of the potential confounding baseline variables had any significant effect on the IBS-SSS. A graph illustrating the relationship between age and baseline IBS-SSS is included in results figure 14.

4.3 Baseline IBS-QOL.

The IBS-QOL actual score is used to calculate the ‘scale score converting it to a score out of 100. All the IBS-QOL scores quoted in this thesis and used in the analysis will refer to the scale score. The overall mean IBS-QOL score at baseline was 52.4, SD ± 20.1. There was no significant difference in the mean IBS-QOL scale score the probiotic group the mean was 53.2 (SD ± 20.2) and in the placebo group 50.7 (SD ± 19.9) (p = 0.43), (table 11). The distribution of baseline IBS-QOL score conforms to the normal distribution and is similar across both the probiotic and placebo group is shown in figures 15 to 17. A model using ordinary linear regression was performed to explore the effects on the baseline IBS-QOL of age, gender, disease subtype

and duration on. None of these factors appear to have any effect (table 12) the linear regression effect of age and the distribution of IBS-QOL are shown in figure 18

A linear regression analysis was performed to explore the relationship between the IBS-QOL and IBS-SSS at baseline. There is a clear relationship between the IBS-SSS and IBS-QOL at baseline with a higher IBS-SSS score (worse symptoms) correlating with a lower IBS-QOL (worse quality of life).

The unstandardised correlation coefficient of the regression is -0.13 (95% CI: -0.151 to -0.10) ($p < 0.001$) (figure 19).

4.4 Laboratory investigations.

At enrolment in the study all patients underwent screening as per the study protocol. Patients with abnormal baseline laboratory parameters and biomarkers were excluded from the study as per the protocol. There were no significant differences between the probiotic and placebo group in any other investigations including liver and renal function tests, thyroid function tests or any of the other investigations. The specific results of these investigations were

not considered to be relevant to the subjects of this thesis and have therefore not been included.

Those patients with an elevated level of the faecal biomarker calprotectin underwent further investigations including colonoscopy and/or wireless capsule endoscopy to establish IBS as the correct diagnosis. Patients in which these investigations yielded alternative diagnoses or reasonable doubt over the diagnosis of IBS were excluded.

The baseline haemoglobin, CRP and faecal calprotectin can be found in table 13 There were no significant differences between the baseline laboratory parameters. These parameters were repeated at the end of the study the values for which are also given in table 13 There were no significant changes in the any of the laboratory parameters measure between baseline and the end of the study or between the probiotic and placebo groups at the end of the study. The results of the small bowel permeability tests found that all but one study participant had normal small bowel permeability at study entry. The overall mean urinary excretion of lactulose/ L-rhamnonose was 0.025 (SD ± 0.020) and did not differ between the probiotic and placebo groups ($p > 0.9$). Only one patient in each group and small bowel permeability test value that was higher than the normal range of 0.06. In view of this result no further analysis of the small bowel permeability was undertaken.

The mean faecal calprotectin in the study cohort at baseline and end of study was 33.1 (SD ± 75.3 µg/g) and 30.1 (SD ± 51.1) which did not differ significantly from each other. The mean faecal calprotectin for the probiotic and placebo groups at baseline were 35.2 µg/g (SD ± 89.3) and 29.1 µg/g (SD ± 38.6); and study completion 29.8 µg/g (SD ± 35.6) and 32.5 µg/g (SD ± 72.1) respectively which also did not differ from one another significantly (table 14).

Twenty two patients had faecal calprotectin levels that were higher than the normal range at study entry (normal ≤50µg/g), mean 113.9 µg/g (SD ± 56.1). The mean IBS-SSS at baseline in patients with a faecal calprotectin of ≥50 was 302.98 and 312.59 in those with a faecal calprotectin of ≤50 which did not differ between the two groups. In this group the post-study calprotectin was 80.9 µg/g (SD ±116.4) also did not differ significantly from the baselines level. In the probiotic group there appears to be a trend in reduction of the faecal calprotectin from 111.4 µg/g (SD ± 62.5) at baseline to 66.6 µg/g (SD ± 49.8) ($p = 0.18$) compared to the placebo group calprotectin as baseline 118.4 µg/g (SD ± 48.7) and post-study 106.8 µg/g (SD ± 194.4) ($p = 0.88$). However, the number of cases at 22 is small and the standard deviation of the results is large for both groups and the difference does not reach significance.

4.5 Safety and tolerability.

4.5.1 Side effects, adverse events and study withdrawals.

No adverse events or serious adverse events were documented during the study. The numbers of reported side effects in the study were relatively few and most were short lived lasting only a few days up to a week. The reported side effects are shown in Table 15. In the probiotic group there were more reported events of nausea and bloating compared to placebo. The probiotic studied is previously known to cause short term nausea and bloating in a small number of patients which is consistent with the findings in this study.

A total of 34 (18.3%) patients withdrew from the trial during the active treatment phase, of these 24 (19.4%) were in the probiotic arm and 10 (16%) in the placebo. 28 (15.1%), (19 (15.3%) probiotic and 9 (14.5%) placebo) of the patients who withdrew did so because of social or personal reasons not related to the study (work commitments, moving away from study area, personal bereavement etc.) or did not attend study appointments and could not be contacted. Of remaining 6 withdrawals, 5 in the probiotic group were due to side effects (diarrhoea and abdominal pain, taste of the product, nausea, flatus, bloating) and 1 in the placebo group (constipation). A further 14 patients, 7 from each group withdrew during the follow up period. This gives a total of 152 patients at week 12 and 138 at week 16 for the PP analysis.

4.5.2 Compliance and tolerability.

The patient compliance with study medication was assessed at regular intervals during the treatment phase of the study as per the study protocol. Compliance with taking study medication was very high throughout the study with >97% of participants in both probiotic and placebo groups missing either none or less than one dose of medication per week (table 16). Of those patients that did not report specific side effects as discussed in section 5.21 the probiotic and placebo were well tolerated.

4.6 Primary and secondary outcomes: Result and analysis of changes in IBS-SSS and IBS-QOL.

All 186 patients were included in the ITT analysis using the LOCF principle for missing data as described. 152 patients were included in the PP analysis at week 12 and 138 at week 16.

4.6.1 Change in IBS-SSS

The mean IBS-SSS scores at week 12 differed significantly between the two groups at 230.07 (SD ± 108.87) in the probiotic and 270.88 (SD ± 103.52) in the placebo group (p = 0.027) in the PP analysis; and 243.18 (SD ± 107.15) probiotic and 277.18 (SD ± 104.47) placebo group (p = 0.044) in the ITT analysis (tables 17 and 18). The primary end-point for the study was the difference in the change in IBS-SSS scores at week 12. The change in IBS-SSS at week 12 in the probiotic group was -70.97 (SD ± 88.25) and -32.02 (SD ± 80.88) in the placebo group in the PP analysis (p = 0.010) and -63.25 (SD ± 85.95) and -28.30 (SD ± 79.89) respectively in the ITT analysis (p = 0.012). The difference in change between the intervention group (probiotic) and placebo group at week 12 was -38.95 (p = 0.01; CI: -68.24 to -9.66) for the PP analysis and -34.95 (p = 0.01; CI: -62.03 to -7.87) in the ITT analysis. The primary end-point analysis is based on one-way ANOVA of the IBS-SSS at week 12 and is significant at the 5% level. There is a small observed difference in magnitude of effect between the PP and ITT analysis but the results remains significant at the 5% level. The LOCF method does not shown any major difference form the per-protocol analysis and can be considered as a crude sensitivity analysis of the imputed values of the LOCF method.

Adjustment of the primary end-point for variance in IBS-SSS at baseline using ANCOVA analysis results in only a very small difference in magnitude of

effect but no difference in the significance. Adjusted mean difference in change of IBS-SSS at week 12 was -39.18 (p = 0.009, CI: -68.24 to – 9.94) and -34.85 (p = 0.012, CI: -61.88 to -7.83) for the PP and ITT analyses respectively.

An ordinary linear regression model was fitted to the IBS-SSS total score at week 12 for both the PP and ITT analysis. The resultant estimated regression coefficient for the PP and ITT analyses showed a mean difference in score between the placebo and treatment of 40.81 (p = 0.027, CI: 9.94 – 68.42) and 34.12 (p = 0.048, CI: 0.36 – 67.87) respectively. This is a basic estimate without adjustments for any potential confounding factors, the magnitude of effect is smaller in the ITT analysis but both are significant and the 5% level. An adjustment for baseline SSS score was made to the regression models. For the PP analysis the estimated coefficient after adjustment for baseline IBS-SSS was 39.18 (p = 0.009, CI: 9.94 – 68.42) and for the ITT 34.85 (p = 0.012, CI: 7.83 – 61.88). In both cases this adjustment for baseline results in a slightly smaller magnitude of effect but the confidence intervals are slightly narrower and there is no observed difference in the significance of the result. A further model adjusting for age was also explored but the effect was not significant and so was removed from the model.

There was no observed difference in the mean IBS-SSS scores at weeks 4 and 8 or at the end of the 4 week follow up period (week 16) in either the PP

or the ITT analysis between the probiotic and placebo groups (tables 19 and 20). The IBS-SSS mean scores for the ITT analysis are illustrated in figure 20.

Secondary end points included changes in the individual IBS-SSS component scores at week 12 and 16. The mean change in pain scores at week 12 were -27.54 (SD ± 47.65) and -11.38 (SD ± 46.22) in the probiotic and placebo groups respectively ($p = 0.048$) for the PP analysis; and -23.40 (SD ± 45.41) and -9.05 (SD ± 46.09), ($p = 0.48$) in the ITT analysis. The mean change in the 'bowel habit satisfaction scores at week 12 were -17.44 (SD 23.31) and -6.78 (SD ± 22.74) in the probiotic and placebo groups respectively ($p < 0.01$) for the PP analysis ; and -14.18 (SD ± 22.73) and -4.86 (SD ± 21.98), ($p = 0.010$) in the ITT analysis. There were no differences in any of the other IBS-SSS components scores at week 12 and no difference in any of the component scores at week 16.The mean values for the component scores and mean change in scores for pain, bloating bowel habit satisfaction and QOL at weeks 12 and 16 are shown in tables 21 to 24. The component scores for the ITT analysis at week 12 are illustrated in figure 21.

4.6.2 Change in IBS-QOL.

A Change in the IBS-QOL was one of the secondary end-points for the study. There were no significant differences in the IBS-QOL score at any time

points in the study. Particularly there was no significant difference in the IBS-QOL at weeks 12 or 16 in either the PP or LOCF analysis (table 25 and 26) as there were no significant changes in the IBS-QOL observed then no further analysis was undertaken.

4.6.3 Sub group and post-hoc analyses.

4.6.3.1 IBS disease sub-type analysis.

The study protocol did not stratify patient inclusion by disease sub-type at study entry. Patients were divided into sub-type according to the Rome III classification of type by predominant stool type. There were no significant differences in the numbers or patients of each sub-type between the probiotic and placebo group (table 6). Sub-group analyses of the primary end point were undertaken on the individual disease sub types but no significant differences were found but the size of the groups were notably small.

4.6.3.2 Analysis of primary end-point based for males and females.

As previously reported there were significantly more females (129) than males (57) in the study cohort but the proportions of each gender are similar within the placebo and probiotic group and consistent with the known

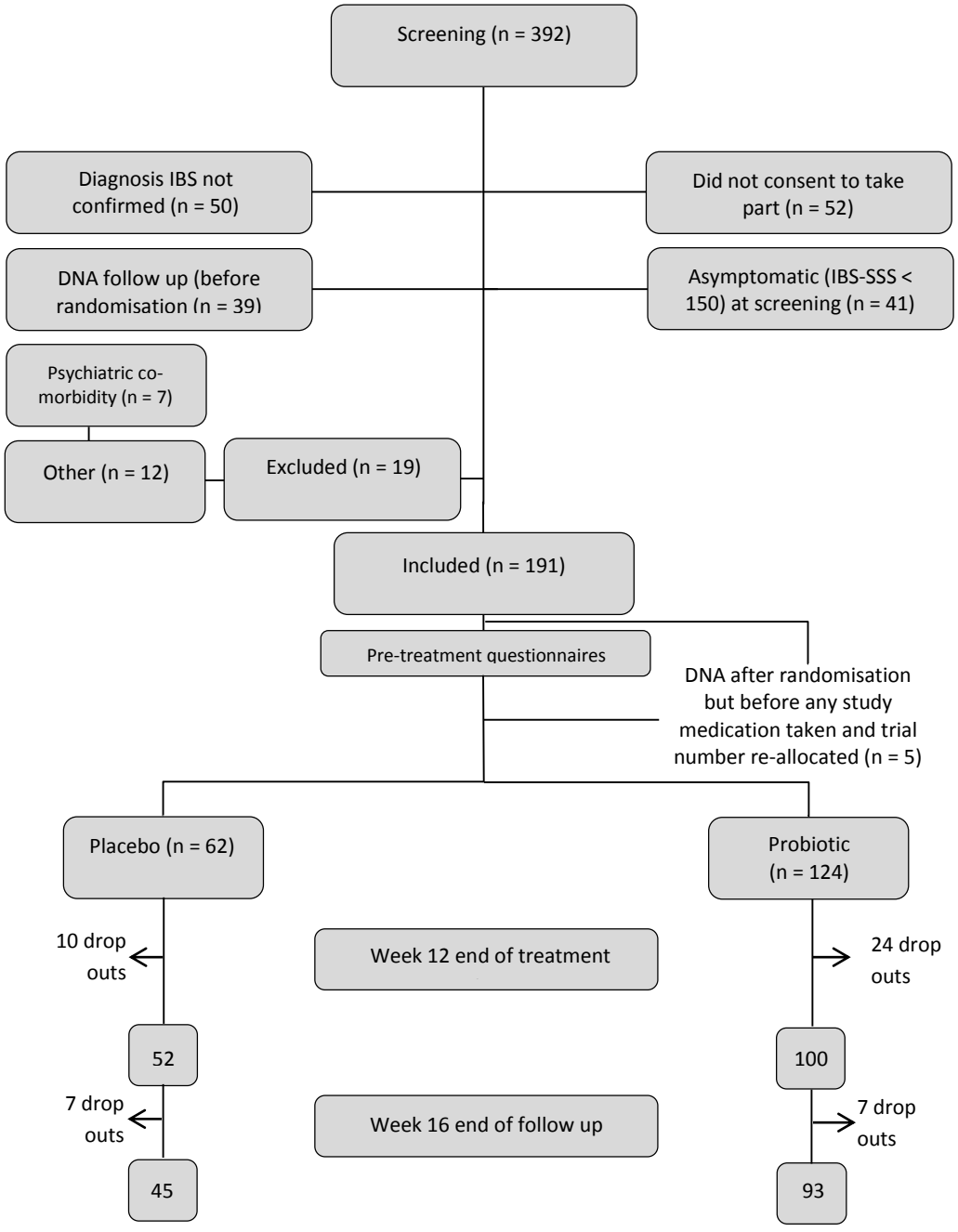
predominance of female patients with IBS in the UK population. A sub group analysis was performed based on gender which shows that the mean change in the IBS-SSS at week 12 (the primary end-point) in the probiotic arm is similar at -71.24 (SD ± 88.24) and -71.65 (SD ± 89.64) in males and females respectively in the PP analysis (p = 0.98) The change in IBS-SSS at week 12 in the placebo arm in both groups is also similar at -31.52 (SD ± 88.24) and -36.84 (SD ± 63.67) respectively (p = 0.83). In the ITT analysis the changes in the IBS-SSS at week 12 are -60.65 (SD ± 84.76) and -59.88 (SD ± 89.48) in the probiotic group (p = 0.96) and; -25.58 (SD ± 86.48) and -37.00 (SD ± 61.65) in the placebo group (p = 0.62) for females and males respectively. The ITT analysis yields similar results to the PP analysis with a smaller magnitude of difference as with previous analyses.

The above analyses demonstrate that there are no significant differences in the magnitude of changes in the primary end-point between the females and male sub groups in either the PP or ITT analyses. When taken as separate subgroups the primary end point of difference in the change in IBS-SSS at week 12 remains significant in the female cohort at -39.71 (p = 0.32, CI: -75.88 to -3.54) in the PP analysis and -35.07 (p = 0.31, CI: -66.95 to -3.18) for the ITT analysis. However, in the sub group of male patients no significant difference is demonstrated in the primary end point is -34.81 (p = 0.17, CI: -85.21 to – 15.60) in the PP analysis and -22.88 (p = 0.34, CI: -70.79 to 25.02) in the ITT analysis.

4.6.3.3 Post-hoc analysis or subjects with mild to moderate symptom severity.

It was noted that the mean IBS-SSS score at baseline was considerable higher than expected with a mean value across the whole cohort of 304.4 (SD ± 71.4). Given this finding a post-hoc analysis was conducted to see whether there was a difference in the number of patients achieving mild or no symptoms (as defined by an IBS-SSS of ≤150 at week 12. A total of 24 (21.05%) patients in the probiotic group compared to 8 (13.79%) in the placebo group achieved IBS-SSS scores of ≤150 at week 12. Whilst this shows that a larger proportion of patients in the probiotic group achieved mild or no symptoms by week 12 this did not reach significance at the 5% level, Pearson Chi (1) = 1.34, p = 0.25.

Figure 10: Study Flow Chart.



Study consort flow chart showing numbers of patients in included/excluded and in the study cohort at each stage of the study protocol

Table 7: Patient demographics

	Total	Probiotic	Placebo	P-value
Age				
range	18-65	18-65	19-63	ns
mean(SD)	38.3 (10.6)	39.1 (10.5)	36.8 (10.7)	
Gender n(%)				
male	57	40 (32.3)	17 (27.4)	ns
female	129	84 (67.7)	45 (72.6)	
Disease duration n(%)				
< 1 yr	13	10 (8.1)	3 (4.8)	
1 – 5 yrs	96	62 (50)	34 (54.8)	ns
>5 yrs	77	52 (41.9)	25 (40.3)	
Disease sub-type n(%)				
IBS-M	66	38 (30.7)	28 (45.2)	
IBS-D	70	48 (38.7)	22 (35.5)	ns
IBS-C	40	31 (25.0)	9 (14.5)	
IBS	10	7 (5.6)	3 (4.8)	
Ethinicity n(%)				
White(British)	104	68 (54.8)	36 (58.1)	
White (Irish)	2	1 (0.8)	1 (1.6)	
White (other)	22	15 (12.1)	7 (11.3)	
Asian (British)	4	4 (3.2)	0 (0)	
Asian (other)	4	2 (1.6)	2 (3.2)	
Black (British)	11	8 (6.5)	3 (4.8)	ns
Black (African)	7	5 (4.0)	2 (8.1)	
Black(other)	6	3 (2.4)	3 (4.8)	
Mixed Race	9	6 (4.8)	3 (4.8)	
Other	5	4 (3.2)	1 (1.6)	
unknown	12	8 (6.5)	4 (6.5)	
Smokers n(%)				
	25	17 (13.7)	8 (12.9)	ns

Demographics of patient cohort including age, gender, duration of disease, IBS subtype (IMBS-M (mixed), IBS-D (diarrhoea), IBS-C (constipation), IBS (unclassified by predominant stool type) and ethnicity. P values given for observed difference between the probiotic and placebo cohort.

Table 8: Baseline medications.

	Total	Probiotic	Placebo
SSRIs (low dose)	15	13	2
Amitriptyline	7	4	3
PPI	18	11	7
Mebeverine	7	6	1
Colpermin	0	1	0
Buscopan	7	5	2
Paracetamol	8	7	1
Codeine	6	5	1
Gabapentin	1	1	0
NSAIDs	4	2	2
Tramadol	4	3	1
Loperamide	7	5	2
Doperidone	1	1	0
Cyclizine	1	1	0
Ranitidine	4	3	1
Laxatives	3	3	0

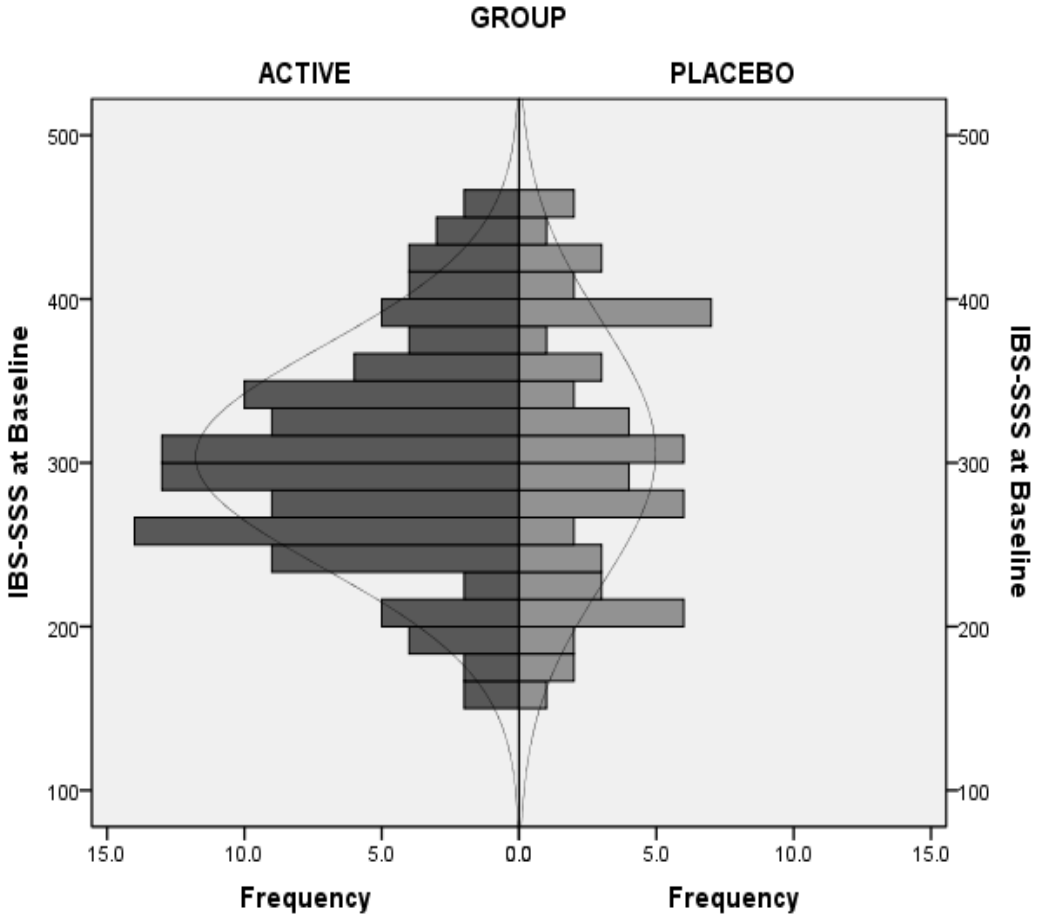
Number and type of medications being used by participants at study entry. Selective serotonin reuptake inhibitor (SSRI), proton pump inhibitor (PPI), non-steroidal anti

Table 9 : Mean IBS-SSS scores at baseline

IBS-SSS	Total	Probiotic	Placebo
Average of week -1 and 0)			
Mean	304.4	303.6 ‡‡	306.0 ‡‡
SD (±)	71.4	68.0	78.2
Range	152 - 456	165 – 456	325.8
Week -1			
Mean	307.6*	305.1 † **	312.5‡ **
SD (±)	71.7	69.1	76.9
Range	152 - 460	152 – 456	152 – 460
Week 0			
Mean	300.9*	302.1† *†	298.5‡ *†
SD (±)	78.5	74.8	85.9
Range	139 – 478	139 - 478	144 - 454

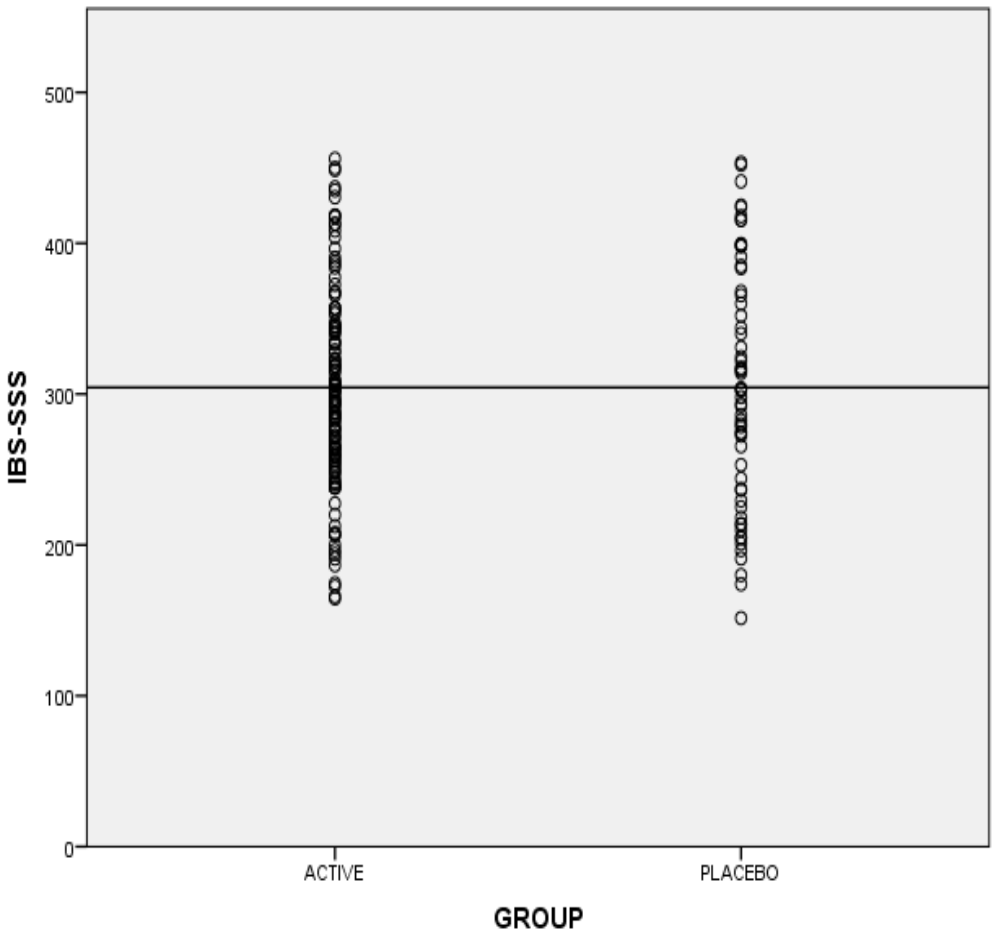
Comparison of IBS-SSS between the probiotic and placebo group at baseline.
* p = 0.067 (CI = -0.460 – 13.685) paired sample t test
† p = 0.479 (CI = -5.599 – 11.860) paired sample t test
‡ p = 0.031 (CI = 1.288 – 25.746) paired sample t test
** p = 0.515 (CI = -30.04 – 15.125) independent sample t test
*† p = 0.776 (CI = -21.408 – 28.618) independent sample t test
‡‡ p = 0.832 (CI = - 24.94 – 20.090) independent sample t test

Figure 11: Distribution of IBS-SSS at baseline (week 0).



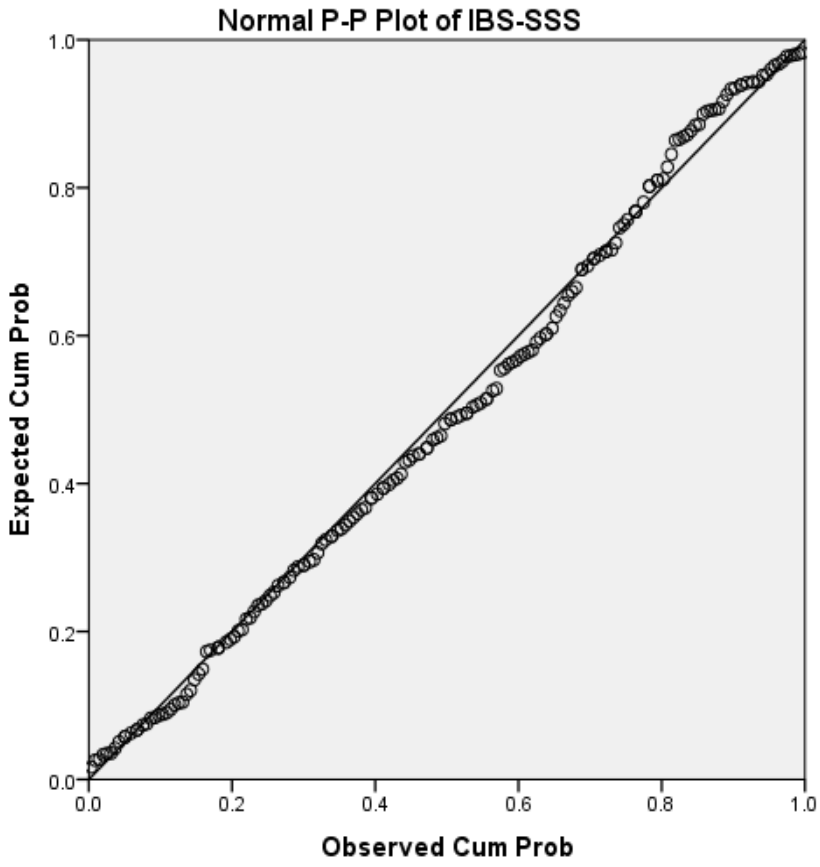
Comparison of frequency distribution of IBS-SSS at baseline between the probiotic groups. The line demonstrates the distribution which conforms to normality in both groups.

Figure 12: Baseline IBS-SSS scatter plot and mean (week 0)



Scatter plot of the IBS-SSS at baseline in both the placebo and probiotic groups. The solid horizontal line depicts the mean IBS-SSS for the entire cohort. Each circle represents an individual patient.

Figure 13: IBS-SSS baseline distribution (P-P plot).



P-P plot (probability-probability) of the baseline IBS-SSS. Demonstrates that the observed and expected (calculated) cumulative probability of the IBS-SSS in the study cohort approximates to the linear and thus to the normal distribution.

Table 10: IBS-SSS at baseline: Model of ordinary linear regression adjusting for baseline confounders.

	Correlation Coefficient	P value	Confidence Interval (95%)	
Mean IBS-SSS (constant)	301.15		238.27	364.02
Age at study	-0.37	0.47	-1.37	0.64
Gender	7.09	0.55	-16.21	30.39
Disease duration	-5.92	0.50	-23.04	11.20
Disease sub-type	7.45	0.19	3.80	18.71

Ordinary linear regression modelling of the base-line IBS-SSS score. The effects of age, gender, disease duration and disease sub-type are demonstrated to have no significant effect on the base-line IBS-SSS.

Figure 14: IBS-SSS: linear regression model for age at baseline

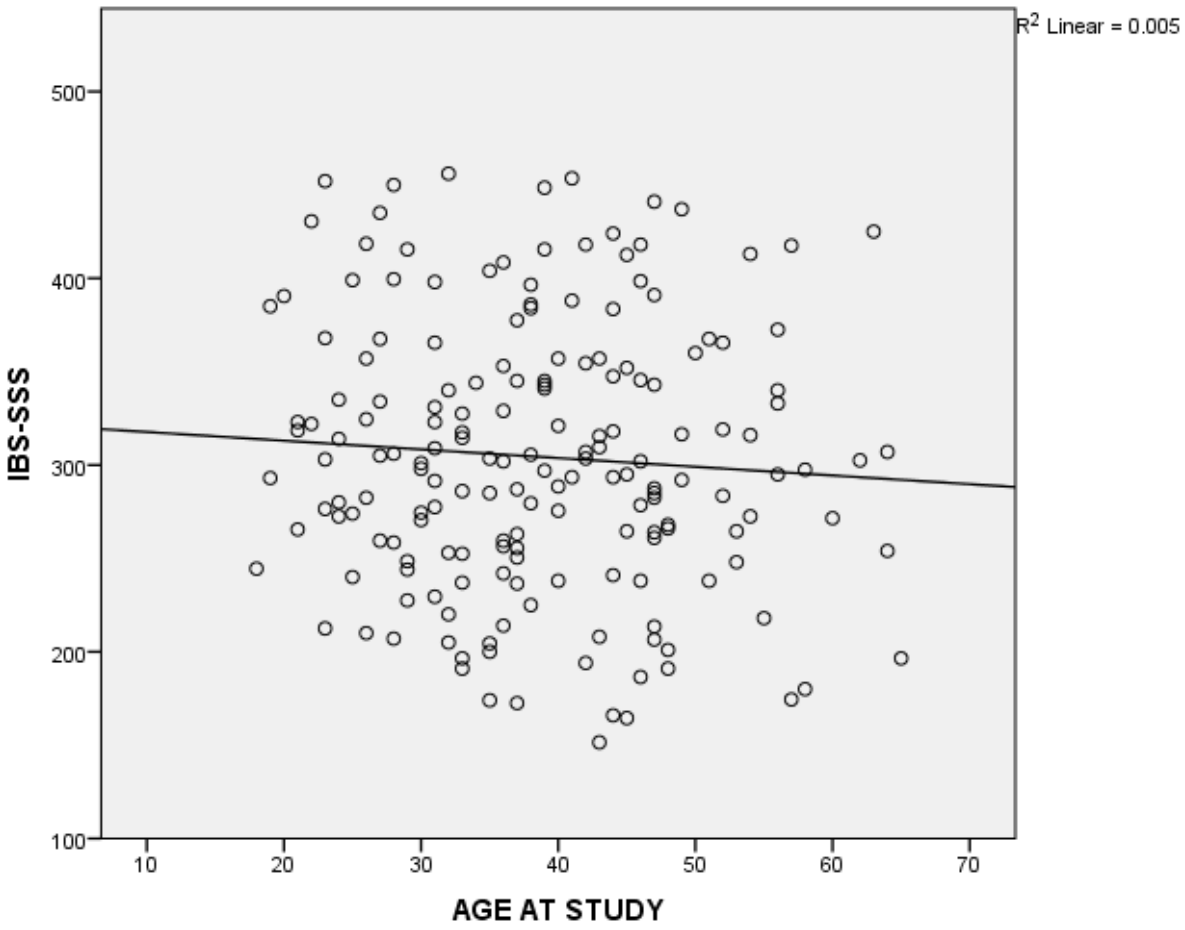


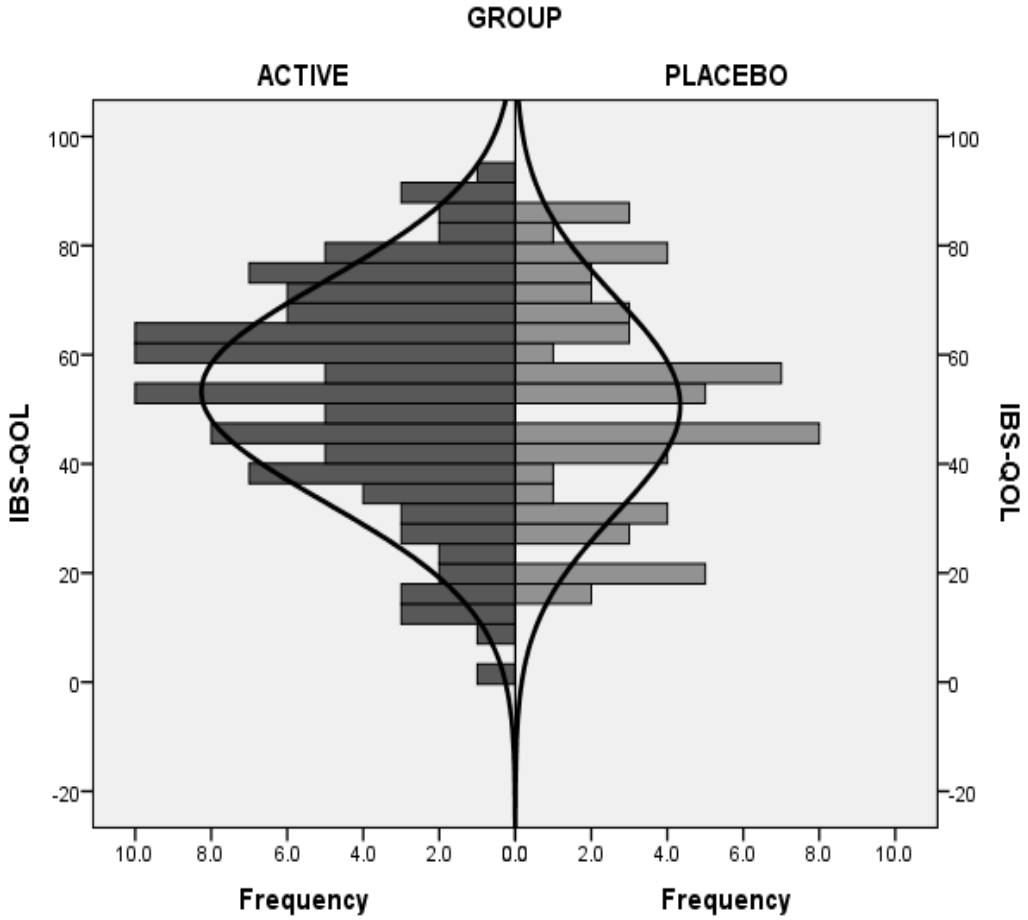
Illustration of the effect of Age on the linear regression model for the IBS-SSS at base-line. Each circle represents and individual patient and the solid line the linear regression modal. Age can be seen to have no significant effect on the model.

Table 11: Mean IBS-QOL scale score at baseline (week 0)

IBS-QOL	Total	Probiotic	Placebo
	Week 0		
Mean	52.4	53.2 *	50.7 *
SD (±)	20.1	20.2	19.9
Range	1 - 93	1 – 93	15 - 87

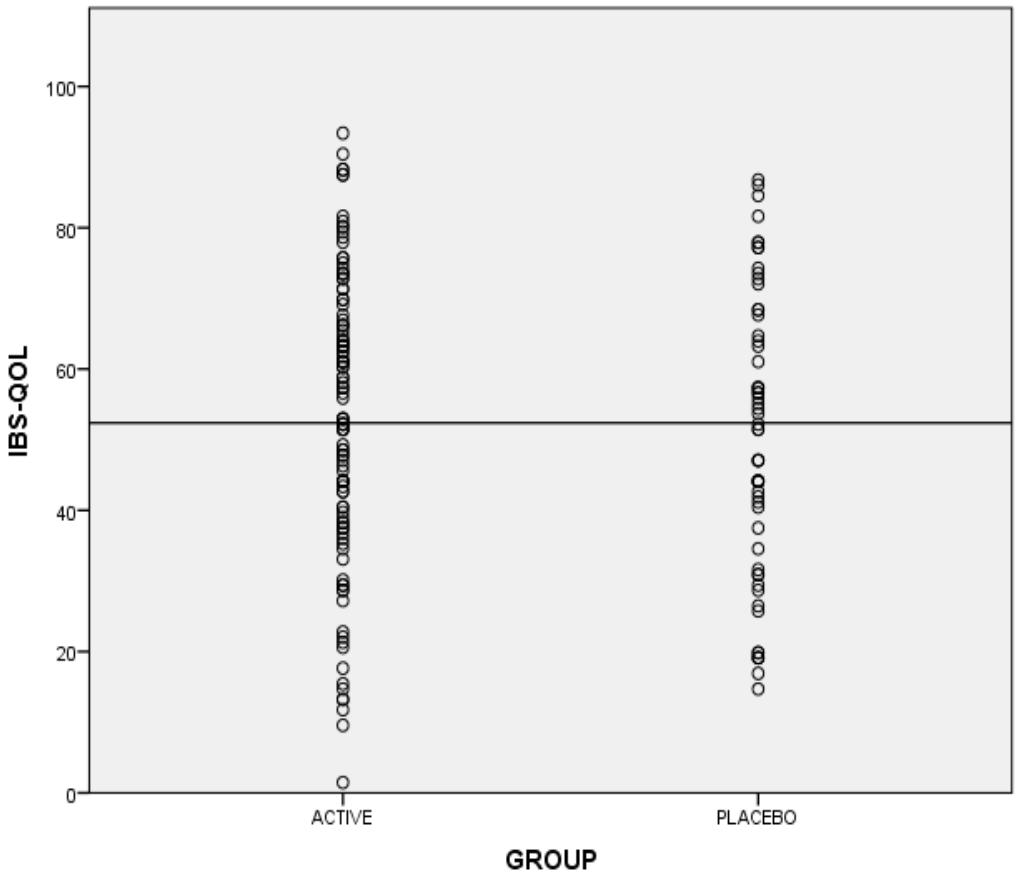
Comparison of the mean IBS-QOL at baseline in the probiotic and placebo groups. * No significant difference in the means p = 0.43

Figure 15: Distribution of IBS-QOL at baseline.



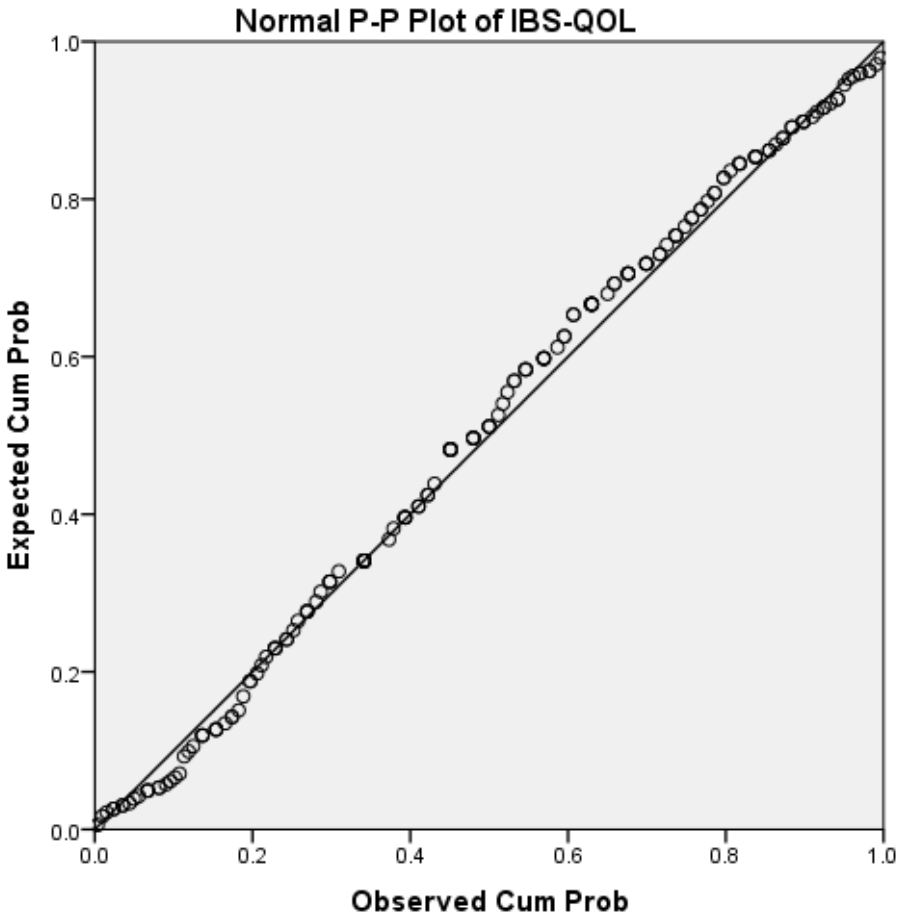
Comparison of frequency distribution of IBS-QOL at baseline between the probiotic groups. The line demonstrates the distribution which conforms to normality in both groups.

Figure 16: Baseline IBS-QOL scatter plot.



Scatter plot of the IBS-QOL at baseline in both the placebo and probiotic groups. The solid horizontal line depicts the mean IBS-QOL for the entire cohort. Each circle represents an individual patient.

Figure 17: IBS-QOL baseline distribution (P-P plot).



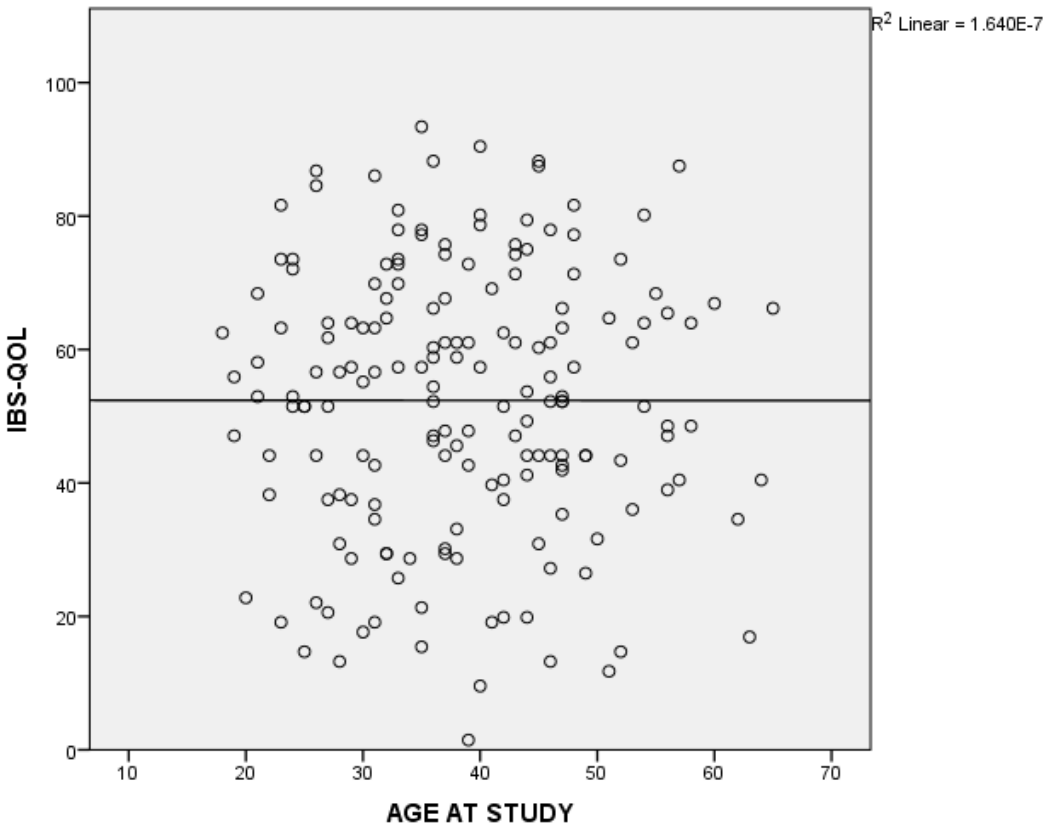
P-P plot (probability-probability) of the baseline IBS-QOL. Demonstrates that the observed and expected (calculated) cumulative probability of the IBS-SSS in the study cohort approximates to the linear and thus to the normal distribution.

Table 12: IBS-QOL at baseline: Model of ordinary linear regression adjusting for baseline confounders.

	Correlation Coefficient	P value	Confidence Interval (95%)	
Mean IBS-QOL (constant)	61.00		43.15	78.84
Age at study	-0.04	0.98	-0.29	0.29
Gender	-1.43	0.67	-8.04	5.18
Disease duration	-2.34	0.35	-7.19	2.54
Disease sub-type	-1.46	0.37	-4.63	1.74

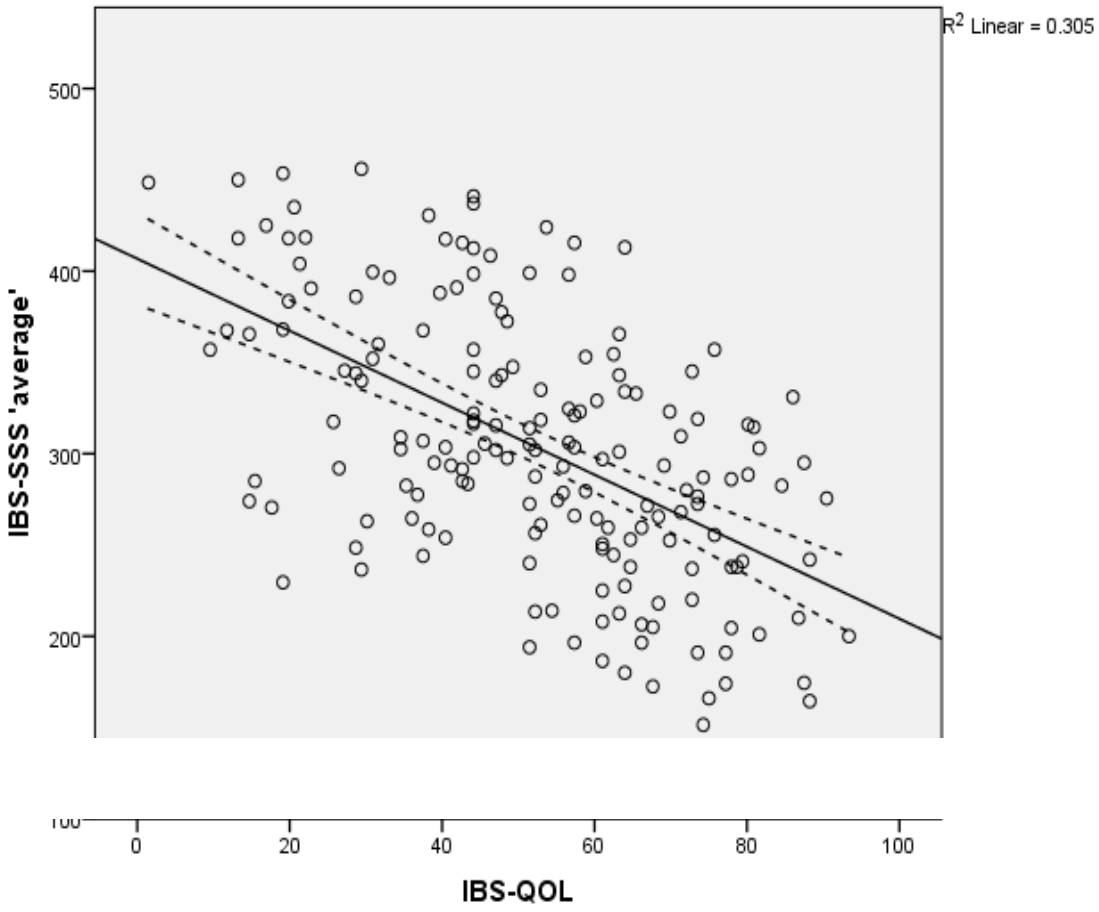
Ordinary linear regression modelling of the base-line IBS-AOL score. The effects of age, gender, disease duration and disease sub-type are demonstrated to have no significant effect on the base-line IBS-QOL.

Figure 18: IBS-QOL: linear regression model for age at baseline



Scatter plot of linear regression of effect of Age on IBS-QOL at baseline. Each circle represents and individual participant. The horizontal line demonstrate the linear regression. Age is shown not to effect baseline IBS- QOL ($R^2 = 1.64 \times 10^{7}$)

Figure 19: Linear Regression analysis of IBS-QOL and IBS-SSS at baseline.



Solid line shows the linear regression (interuppted lines show 95% confidence interval of means).

Linear regression of relationship between the baseline IBS-SSS and IBS-QOL. The solid line demonstrates the linear regression with the interrupted lines showing the 95% confidence interval. A clear linear relationship is demonstrated with worse symptoms (high IBS-SSS) being associated with low IBS-QOL (worse quality of life). R^2=0.305

Table 13: Laboratory parameters at baseline and at study completion

	Overall mean (SD)		Probiotic (mean (SD)	Placebo mean (SD)	P value
Haemoglobin (g/dl)					
Pre-study	13.6 (±1.2)	P = 0.65	13.5 (±1.1)	13.6 (±1.4)	0.70
Post-study	13.5 (±1.2)		13.5 (±1.2)	13.5 (±1.2)	0.96
CRP (ng/l)					
Pre-study	33.1 (±75.3)	P = 0.76	6.0 (±4.4)	7.0 (±9.4)	0.42
Post-study	30.1 (±51.1)		6.9 (±12.4)	5.9 (±4.9)	0.52
Faecal calprotectin (µg/g)					
Pre-study	33.1 (±75.3)	P = 0.87	35.2 (±89.3)	29.1 (±38.6)	0.57
Post-study	30.1 (±51.1)		29.8 (±35.6)	32.5 (±72.1)	0.48

Table 14: Change in faecal calprotectin in participants who had abnormal baseline levels (≥50 µg/g) (n=22)

	Overall mean (SD)		Probiotic (mean (SD)	Placebo mean (SD)	
Faecal calprotectin (µg/g)					
Pre-study	113.9 (± 56.1)	P = 0.32	111.4 (± 62.5)	118.4 (± 48.7)	p = 0.66
Post-study	80.9 (± 116.4)		66.6 (± 49.8)	106.8 (± 194.4)	P = 0.56
			P = 0.18	P = 0.88	

Tables 13 shows the pre- and post-study laboratory parameters (Hb, CRP and Faecal calprotectin).

Table 14 shows the change in the faecal calprotectin from baseline.

Table 15: Reported Side effects.

Side effect	Total	Active	Placebo
Nausea	5	5	0
Weight gain	1	1	0
Change in bowel habit	12	6	6
Bloating	12	10	2
Heartburn/dyspepsia	4	3	1
Pain	7	3	4
Vomiting	3	2	1
Acne	2	1	1
Headache	2	1	1
Vaginal candida	1	1	0
Flatulence	3	2	1
Halitosis	1	0	1
Fatigue	1	0	1
Total	**54**	**35**	**19**

Individual side effects reported by participants during the study period. Each side effect is reported separately (by episode). Some individuals reported multiple side effects which are each reported as an individual episode in this table.

Table 16: Medication compliance

Doses missed	Total (%)	Probiotic (%)	Placebo (%)
Week 4			
Never missed	120 (67.4)	81 (68.6)	39 (65.0)
Missed <1/week	55 (30.9)	34 (28.8)	21 (35.0)
Missed 1-3/week	3 (1.68)	3 (2.5)	0 (0)
Missed >3/week	0 (0)	0 (0)	0 (0)
Week 8			
Never missed	100 (61.1)	64 (58.7)	36 (66.7)
Missed <1/week	60(36.8)	42 (38.5)	18 (33.3)
Missed 1-3/week	3 (1.84	3 (2.8)	0 (0)
Missed >3/week	0 (0)	0 (0)	0 (0)
Week 12			
Never missed	97 (63.8)	63 (63)	34 (65.4)
Missed <1/week	52 (34.2)	35 (35)	17 (32.7)
Missed 1-3/week	3 (2.0)	2 (2)	1 (1.9)
Missed >3/week	0 (0)	0 (0)	0 (0)

Table showing the reported compliance during the study. Compliance with medication was reported by asking individuals at each visit schedule whether they have been compliant with the prescribe study medication.

Table 17: Mean IBS-SSS scores at weeks 4,8,12, and 16 (PP analysis)

	Probiotic		Placebo		P value
	Mean	SD	Mean	SD	
Week 4	260.42	93.29	263.23	101.42	0.859
Week 8	240.42	111.68	259.94	96.94	0.283
Week 12	230.07	108.87	270.88	103.52	0.027
Week 16	246.60	114.74	238.93	104.96	0.706

Table 18: Mean IBS-SSS scores at weeks 4,8,12, and 16 (ITT analysis)

	Probiotic		Placebo		P value
	Mean	SD	Mean	SD	
Week 4	263.53	91.67	272.45	106.82	0.561
Week 8	248.70	108.87	266.72	98.18	0.281
Week 12	243.18	107.15	277.18	104.47	0.044
Week 16	259.73	110.61	262.57	111.37	0.872

Table 17 and 18 show the mean IBS-SSS at each recorded time point during the study for both the PP and ITT analysis

P values calculated using one way ANOVA

Table 19: Change in mean IBS-SSS scores from baseline at weeks 4,8,12, and 16 (PP analysis)

	Probiotic		Placebo		P value
	Mean	SD	Mean	SD	
Week 4	-42.83	74.39	-35.25	80.35	0.548
Week 8	-63.00	92.02	-43.05	84.49	0.191
Week 12	-70.97	88.25	-32.02	80.88	0.010
Week 16	-52.25	96.89	-57.23	86.26	0.861

Table 20: Change in mean IBS-SSS scores from baseline at weeks 4,8,12, and 16 (ITT analysis)

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Week 4	-40.02	71.1	-33.55	78.64	0.578
Week 8	-54.88	88.51	-39.28	85.41	0.261
Week 12	-63.25	85.95	-28.30	79.89	0.012
Week 16	-43.84	92.54	-43.43	82.81	0.977

Tables 19 and 20 show the mean (and standard deviation) changes in the IBS-SSS at each recorded time point in the study. P values calculated using one way ANOVA

Figure 20: Mean IBS symptoms severity score at weeks

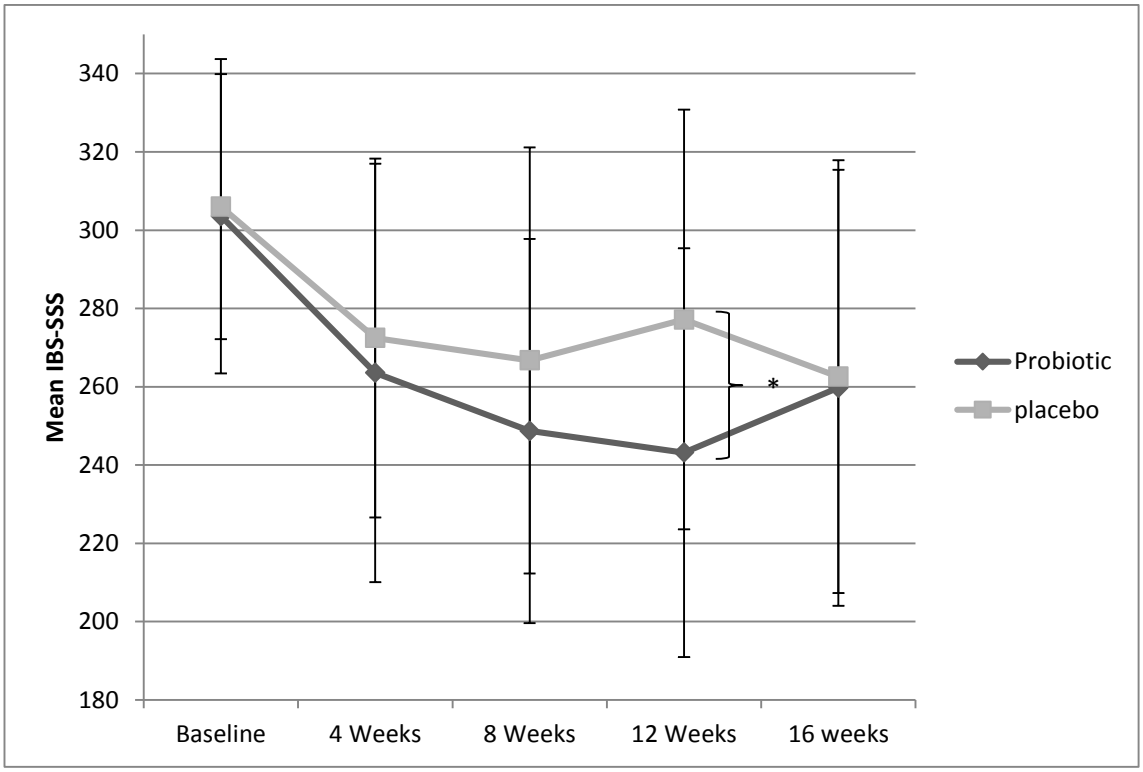


Illustration of the change in the mean IBS-SSS score at each recorded time point in the study. The squares represent the placebo and the diamonds the probiotic groups. The error bars represent the standard deviation. * P = 0.044 (one-way ANOVA)

Table 21: Mean IBS-SSS symptom component scores at weeks 12 and 16 (PP analysis)

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Pain component score					
Week 12	77.72	56.58	102.48	54.09	0.010
Week 16	89.45	87.15	89.78	56.15	0.975
Bloating component score					
Week 12	42.59	29.02	46.81	28.39	0.393
Week 16	44.11	32.76	38.60		0.354
Bowel habit satisfaction component score					
Week 12	55.92	23.76	61.78	17.93	0.120
Week 16	57.22	25.01	55.53	20.24	0.695
QOL component score					
Week 12	53.84	23.58	59.81	20.50	0.124
Week 16	55.83	24.09	55.02	22.02	0.850

Mean individual component scores of the pain, bloating, bowel habit satisfaction and the QOL component of the IBS-SSS at the week 12 (end of treatment) and week 16 (end of follow up) for the PP analysis. P values calculated using one way ANOVA

Table 22: Mean IBS-SSS symptom component scores at weeks 12 and 16 (ITT analysis)

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Pain component score					
Week 12	82.48	55.06	103.20	54.49	0.18
Week 16	92.83	55.35	97.78	58.12	0.58
Bloating component score					
Week 12	44.74	29.36	49.93	29.27	0.26
Week 16	46.82	31.76	45.77	33.10	0.84
Bowel habit satisfaction component score					
Week 12	59.42	23.80	63.87	18.75	0.21
Week 16	61.02	24.53	60.62	21.15	0.91
QOL component score					
Week 12	56.09	23.25	61.67	21.39	0.12
Week 16	58.15	23.50	59.37	22.80	0.74

Mean individual component scores of the pain, bloating, bowel habit satisfaction and the QOL component of the IBS-SSS at the week 12 (end of treatment) and week 16 (end of follow up) for the ITT analysis. P values calculated using one way ANOVA.

Table 23: Change in mean IBS-SSS symptom component scores from baseline at weeks 12 and 16 (PP analysis)

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Pain component score					
Week 12	-27.54	47.65	-11.38	46.22	0.48
Week 16	-15.89	55.81	-18.92	56.10	0.77
Bloating component score					
Week 12	-10.38	23.59	-11.27	24.04	0.33
Week 16	-8.68	24.72	-6.38	24.75	0.57
Bowel habit satisfaction component score					
Week 12	-17.44	23.31	-6.78	22.74	<0.01
Week 16	-15.89	25.02	-12.69	23.96	0.48
QOL component score					
Week 12	-16.03	20.55	-11.42	15.80	0.16
Week 16	-13.78	20.64	-14.36	18.11	0.87

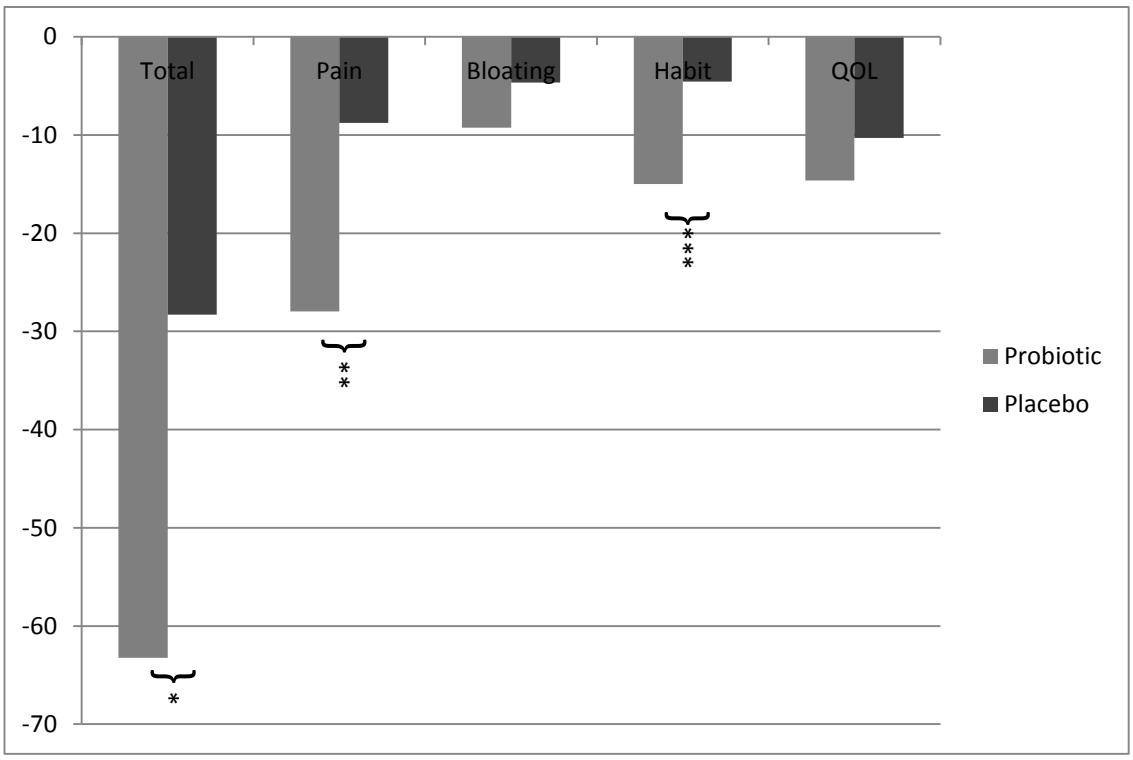
Change in the individual IBS-SSS component scores for Pain, bloating, bowel habit satisfaction and the QOL component for baseline at week12 (end of treatment) and week 16 (end of follow up), PP analysis. P values calculated using one way ANOVA

Table 24: Change in mean IBS-SSS symptom component scores from baseline at weeks 12 and 16 (ITT analysis)

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Pain component score					
Week 12	-23.40	45.41	-9.05	46.09	0.048
Week 16	-13.05	51.88	-14.47	52.09	0.86
Bloating component score					
Week 12	-9.01	22.99	-4.96	23.11	0.27
Week 16	-6.94	24.33	-9.13	22.27	0.56
Bowel habit satisfaction component score					
Week 12	-14.18	22.73	-4.86	21.98	0.10
Week 16	-12.58	24.09	-8.11	23.30	0.24
QOL component score					
Week 12	-14.03	20.00	-8.97	18.49	0.10
Week 16	-11.97	19.94	-11.27	19.13	0.82

Change in the individual IBS-SSS component scores for Pain, bloating, bowel habit satisfaction and the QOL component for baseline at week12 (end of treatment) and week 16 (end of follow up), ITT analysis. P values calculated using one way ANOVA

Figure 21: Change in IBS-SSS overall score and component scores at week 12



Graph showing a comparison of the change in the overall IBS-SSS and individual component scores (pain, bloating bowel habit satisfaction and QOL component) between the probiotic and placebo group at week 12 for the ITT analysis. The overall change in the IBS-SSS at week 12 was the primary end point for the study. P values calculated using one way ANOVA. * p = 0.01, ** p = 0.48, *** p = 0.10.

Table 25: Mean IBS-QOL and change in IBS-QOL at weeks 12 and 16 (PP analysis).

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Mean IBS-QOL Score					
Week 12	60.45	21.56	54.68	20.53	0.114
Week 16	63.12	22.90	58.23	22.33	0.233
Mean change in IBS-QOL score					
Week 12	9.06	18.24	7.67	19.13	0.662
Week 16	13.34	19.27	12.52	19.49	0.815

Table 26: Mean IBS-QOL and change in IBS-QOL at weeks 12 and 16 (ITT analysis).

	Probiotic		Placebo		P value
	Mean	**SD**	**Mean**	**SD**	
Mean IBS-QOL Score					
Week 12	59.85	21.12	55.95	20.65	0.245
Week 16	61.92	22.13	57.73	22.21	0.237
Mean change in IBS-QOL score					
Week 12	6.62	13.22	4.58	13.67	0.343
Week 16	9.69	14.55	6.36	14.39	0.317

Table 25 (PP) and 26 (ITT) show the mean IBS-QOL scores at week 12 (end of treatment) and week 16 (end of follow up); and the mean change in the IBS-QOL at the same time points for the PP and ITT analyses.

Chapter 5

Discussion

The aim of this research study was to undertake a robust and rigorous single centre, randomised, double blind placebo controlled trial to establish the efficacy and safety of a novel multi-strain probiotic in the treatment of patients with symptomatic IBS which has essential been achieved.

Recruitment of patients to the study was slower than initially anticipated and the total study period was therefore extended from 24 to 36 months. It was the initial intention in the study protocol to recruit patients that were directly known to, or referred to the gastroenterology outpatient department at Kings College Hospital. However, the recruitment protocol was amended, after appropriate submission and ethical approval of the new protocol. This alteration allowed for direct recruitment of patients with symptomatic IBS from primary care physicians. This alteration not only supported recruitment to the study but also helped ensure that the cohort of patients gave a broader reflection of the overall local population of patients with symptomatic IBS by including patients from primary healthcare providers that otherwise may not have been able to access the study.

The impact of including patients from both primary and secondary care on the observed efficacy of the probiotic/placebo was not considered as part of the original study design. It is probably reasonable to assume that those patients with IBS who are referred to secondary care may have more severe symptoms. However, it has also been previously established by other authors that significant differences in psychological profiles exist between consulting

and non-consulting patients, [57] and that psychological co-morbidities are more prevalent in patient who report more numerous or more severe symptoms. [7] In considering the impact of inclusion of both sub-populations it's difficult to theorise whether those participants included from a primary care background have symptoms that are less severe and so are more likely to be responders. It is equally difficult to surmise whether the differing psychological profiles in either sub-populations make it more or less likely that they will be actual responders or be likely to report a placebo effect.

The inclusion of the participants from both sub-populations arguable makes the results relevant and applicable across both populations, however, given that the study design was not powered to demonstrate efficacy in the sub-populations is not possible to prove or refute this assumption.

Overall, nearly four hundred patients were screened for inclusion in the study in order to achieve the required cohort of 186 patients specified by the study power calculation. Initially, the exclusion of greater than 50% of patients in the screening process may lead to suspicion that the exclusion criteria for the study were too specific resulting in a study cohort that is too narrowly defined and not representative of the intended target population. However, more careful inspection of the excluded patients clearly demonstrates that only 19 patients with a diagnosis of IBS were excluded from the study because of ineligibility. The remaining exclusions can be seen in the CONSORT flow chart (figure 10) were as a result of a combination of reasons that included the patient not

currently having any active symptoms; not having a diagnosis of IBS; declining to give consent; and failing to attend the second screening visit.

The screening process described in the protocol consisted of 2 visits one week apart. Whilst not formally described as a ‘run in period’ this allowed for two separate measurements of the IBS-SSS to ensure some stability of the baseline data for study analysis. However, as a result of this screening protocol five patients who were allocated a trial identification number after being screened at ‘visit one’ subsequently did not attend ‘visit 2’ to complete the. Despite repeated attempts to contact these patients they were essential lost to follow up and took no further part in the study. Blinding of the allocation of these 5 patients and their associated trial ID number was maintained and none received any study medication. It was therefore decided that reallocation of these trial numbers would preserve the size of the study cohort without undermining the integrity of blinding of the ITT principle. There were no breaches of the randomisation and/or blinding protocol during the study period.

It is well established and accepted that IBS symptoms in individual patients show a high degree of temporal variability. It has also been suggested that inclusion of a period of baseline observation in clinical trials of IBS populations is an important consideration in study design[258] and this approach has been adopted by multiple research groups two of which are cited as examples. [29, 30] During the screening period of our study some variability

in the IBS-SSS score at baseline was identified. In the probiotic group no significant temporal variability was noted but in the placebo group the variability was statistically significant. There were no differences in the mean IBS-SSS score between the probiotic and placebo group at either time. However, after consideration it was decided that the most appropriate action was to average the IBS-SSS scores from week -1 and week 0 to mitigate this variability in the placebo group. The averaging of baseline was conducted in both probiotic and placebo groups for continuity which remained similar after averaging.

The decision to include twice the number of patients in the active treatment group compared to placebo may result in some criticism of the study design. This decision was pre-specified in the protocol and considered in the power calculation and therefore does not affect the statistical validity of the study's findings. Prior to the commencement of this study there was no formal evidence or previous clinical trial to demonstrate the safety and/or tolerability of the probiotic being studied. This is despite it being used, albeit, in relatively small numbers in 'consumers' and volunteers with a variety of gastroenterological conditions including IBS. When designing the study it was therefore considered essential that this study yield sufficient information regarding side effects, adverse events and overall safety and tolerability of the study and hence the decision to increase the size of the probiotic cohort. This also increases the recruitment as patients have a more than average chance of being on the potential beneficial agent.

The pre-study power calculation indicated that a study cohort of 186 patients would be adequate to demonstrate a difference in the primary end point with 90% power. However, when conducting the study analysis it was identified that the original power calculation was performed using a one-sided or directional test to calculate power. Utilising a one sided tests makes the incorrect assumption that the change in the primary end-point is directional. Furthermore, the original power calculation utilised an MCID of ≥50 points in the IBS-SSS to define a responder whereas the study primary end-point was the difference in the mean change in score rather than utilising a categorically defined end point. The decision to amend the study protocol and change the primary end-point to a comparison of means was undertaken prior to starting recruitment and was felt to be more appropriate primary given the exploratory nature of the study. As a result of this methodological error the power was recalculated using a comparison of the means and a non-directional or two-sided test resulting in a calculated study power of the actual study of 80% which remains adequate. The latter power calculation dose not utilise the 50 point MCID. This methodological correction has not had a significant adverse effect on the power of the study or the validity of the outcome but should still be noted.

We have already demonstrated that there were no significant differences in the baseline demographics between the probiotic and placebo arms of the study. The study cohort does include significantly more females than males but

the gender ratio of 2.26:1 (female: male) is reflective of the known female predominance in the study population and is similar in both arms of the study. This gender imbalance could have been addressed by stratification of recruitment by gender but this was not included in the study design. Whilst the lack of stratification has resulted in a cohort that is reflective of the gender characteristics of the IBS population it has led to the inclusion of only a relatively small cohort of men (57) in this study. It was never the intention to power the study sufficiently to demonstrate efficacy in each gender separately but this lack of power if more keenly seen in the male cohort due to its size. The impact of this is discussed in further details later.

There were no adverse events or serious adverse events recorded during the study period and the side effects reported were mild and transient in nature. It is noted that in the first few days of taking the probiotic there was a higher number of patients who reported an increase in bloating but this settled after a few days in all but a few cases. It has been previously noted by the developers/manufacturers of the studied probiotic that some bloating does occur during the first few initial days of treatment. There is evidence that bacterial fermentation by luminal bacteria may contribute to bloating both through a methanogenic process [225] and changes in colonic motility. [229] It is therefore possible that this transient bloating may be as a result of changes to the luminal microbiome that occur as a result of the introduction of the probiotic. The underlying physiological mechanisms that lead to this transient bloating for

this particular probiotic preparation has not been established and would require further research that is not the subject of this thesis.

Withdrawals from the study were less than predicted at 18.3% during the treatment phase with a further 5.6 % withdrawing during the follow up period. Of the withdrawals from the study only 5 (4%) of patients in the active treatment group can be directly attributed to side effects of the probiotic. Compliance with study medication was very high with the vast majority of patients missing none, or less than one dose per week on average. The method of assessing compliance was to simply ask each patient directly at each study visit. Alternative methods of assessing compliance by measuring the amount of remaining probiotic at each visit were considered too difficult and equally unreliable for this study. It is accepted that relying on patient reporting of compliance may not give a true reflecting of the actual compliance. It is of note that compliance in both the probiotic and placebo arms of the study was similar.

Overall, the number and nature of side effects and the tolerability of the probiotic observed in this study is reassuring. The apparent lack of significant of severe side effects observed in the active treatment arm of this study is important. Many currently available, conventional medications for the treatment of IBS are reported to have a variety of sometimes quite significant side effects which can limit their usefulness in clinical practice. Whilst the observed safety

profile in this study indicates that the probiotic can be used without any significant concerns this should be corroborated by further studies.

The primary end point of this study; a difference in the change in IBS-SSS score between the placebo groups at week 12 was achieved with a highly significant result ($p = 0.012$) and an 80% power suggesting that this probiotic preparation is efficacious at improving symptoms in patients with IBS. In addition, there was a significant difference in the actual IBS-SSS score between the 2 groups at week 12 ($p = 0.027$ (PP) and $p = 0.044$ (ITT)). Changes in the IBS-SSS at earlier time points during the study were not significantly different between the probiotic and placebo groups. The trend in IBS-SSS shows a continuous reduction in global symptom severity throughout the study in the probiotic group with a static response in the placebo group. Previous empirical information from the probiotic manufacturer had indicated that a 12 week treatment period would be appropriate to demonstrate efficacy which appears to be supported by our study.

The changes observed in the IBS-SSS component scores are only significant for pain and bowel habit satisfaction. It is acknowledged that this study was not powered to detect changes in the individual component scores. Furthermore, the design of the IBS-SSS instrument includes a pain score that is weighted higher than the other component scores. Our study was therefore perhaps more likely to show a significant difference in the pain component

score given as the magnitude of change is also likely to be larger as a result of this weighting. Regardless of this, in our study the observed change in IBS-SSS score appears in principle to be due to changes in the component scores for pain and satisfaction with bowel habit. The significant change in the pain component score is worthy of note as pain is perhaps considered to be the singularly most important symptom of IBS,[255] and is the only absolute symptom required by the Rome III criteria. A further larger study, with adequate power may clarify whether improvement in each individual component symptoms scores contributes to the overall improvement in global symptom severity.

Only 3 RCTs of probiotics in IBS have been identified that utilise the IBS-SSS instrument as an outcome measure, two of which are only available as abstracts. The first of these studies by Simren et al. compared a dairy preparation containing *Lactobacillus paracasei, Lactobacillus acidophilus* and *bifidobacterium lactic 299v* in 67 patients. In this study both the treatment group and placebo group showed a significant reduction in the IBS-SSS score from baseline ($p = 0.006$ and $p = 0.0001$) respectively but there was no difference between the probiotic and placebo groups.[259] A further study by the same group in 58 patients, used a non-dairy liquid preparation containing the same strain of bifidobacteria; showed a significant change in IBS-SSS from baseline in the placebo group ($p = 0.0001$) but not in the probiotic group $P = 0.08$).[31] Niv et al. also used the IBS-SSS for the primary end-point in a study of *lactobacillus reuteri ATCC 55730* in a study of 57 patients but whilst they

demonstrated an improvement in the overall IBS-SSS across the cohort there was no observed difference between the probiotic and placebo groups.[33] All three of these studies had relatively small study cohorts and do not report whether a power calculation was performed prior to commencement of the study. Both of these factors may explain the apparent lack of efficacy demonstrated although it is equally conceivable that the probiotics used were actually ineffective.

In the meta-analysis by McFarland et al. the pooled relative risk (RR) was 0.77 (85% CI 0.62 to 0.94). NNT 7.3; for reduction in global symptoms after treatment with probiotic compared to placebo using a random effect model, heterogeneity X^2 = 41.0 d.f. [32] This meta-analysis indicates that in the studies that included a measure of global symptom severity in their design there is an apparent reduction in global symptoms after treatment with probiotic compared to placebo. However, out of the 14 studies that reported changes in global symptom severity only 4 studies used specific, independently validated symptom severity scores. One of the largest studies of probiotics in IBS by Whorwell et al. assessed the efficacy of *Bifidobacterium infantis 35624* in 362 females with IBS in a dose ranging study. They reported a significant difference in global symptom severity as measured by their own composite score at the end of treatment (p = 0.013)[243] which is comparable to our results. It should be noted though that this study did not include male patients and excluded those patients with the most severe pain scores.

A further secondary end-point was the maintenance of a difference in the change in the IBS-SSS scores at week 16 but the study did not demonstrate a significant difference between the placebo and probiotic for this outcome. The purpose of this end-point was to explore whether the therapeutic effect of treatment with the probiotic preparation was maintained four weeks after stopping treatment. Whilst the study did not demonstrate this the actual results for the probiotic and placebo groups at this time point are interesting and are worthy of further consideration. The results from the probiotic group show a mean change in IBS-SSS at week 12 of -70.97 (SD ± 88.25) at week 12 and -52.25 (SD ± 96.89) at week 16 in the PP analysis. At the same time points in the placebo group the mean change is -32.02 (SD ± 80.88) and -57.23 (SD ± 86.26) respectively. The PP analysis is chosen specifically as the ITT analysis utilises the LOCF principle to generate missing data which may result in a false elevation of the 'end of follow up' value. When considered individually it would appear that the therapeutic effect in the probiotic group is maintained after four weeks albeit with some reduction in magnitude of effect. In the placebo group something different occurs and there is a considerable increase in the reduction in the IBS-SSS score four weeks after stopping the placebo. The reason for this change in the IBS-SSS in the placebo group is not clear. One possible explanation is that the placebo itself led to a reduction in the change in IBS-SSS which, whence the placebo was withdrawn then reversed. The placebo consisted of sterile water with inert natural flavourings and colourings as described in the method. Given that it contains only very small amounts of

ascorbic acid and beta carotene at it is highly unlikely for this to be the case. Furthermore, an improvement in the IBS-SSS score during the treatment phase was observed in the placebo group and it therefore cannot be concluded that the placebo led to a worsening of symptoms. The reason for this behaviour in the placebo response is not apparent. Therefore, it would be reasonable to conclude that whilst no significant difference is demonstrated in the change in IBS-SSS between the 2 groups at week 16 there does appear to be at least some maintenance of the therapeutic effect of the probiotic. Further research is needed to examine the potential maintenance of the therapeutic effect.

The final secondary end-points that need some discussion are the change in the IBS-QOL at weeks 12 and week 16. The IBS-QOL was specifically included in this study as an end-point to explore whether an improvement in the IBS-SSS corresponded to an improvement in the perceived quality of life of patients. This study did not demonstrate any difference between probiotic and placebo at any time point for the IBS-QOL score. At baseline the IBS-QOL is similar between the 2 groups and perhaps even more importantly there is a clear relationship between the IBS-QOL and IBS-SSS, unstandardised regression coefficient of -0.13 ($p = 0.001$). As expected, this demonstrates that worse symptoms are associated with a poor quality of life score. This relationship, which would seem to be a logical, it is not continued at the end of the intervention. One explanation could be that a change in symptom severity occurs quicker in response to treatment than a subsequent subjective change in QOL and therefore this study was not long enough to detect the

latter. However, whilst this may appear possible there is no evidence to currently support this theory. It is also possible that as this study was not powered to show a specific change in the IBS-QOL score the lack of apparent efficacy to change QOL occurs simply as a result of a type II error.

The IBS-QOL itself was constructed to be a reliable tool to evaluate disease specific QOL in patients with IBS and was validated for internal validity and reproducibility by the original authors.[17] However, whilst addressing whether changes in the IBS-QOL were representative of changes in response to treatment the authors did not address whether such changes corresponded with global symptom change. The construct of the IBS-QOL questionnaire and the separate domains within it deal primarily with ideas that could be considered to be of a psychological nature (dysphoria, sexual dysfunction, body image, social interaction and relationships). Moreover, in a later article, one of the leading authors from the original article concludes that changes in the IBS-QOL are demonstrated primarily in the psychosocial rather than physical domains.[260] It is therefore possible to theorise that changes in the IBS-QOL may not correspond to changes in physical symptoms although this would need to be tested further and is not part of the remit of this study. It one possible implication is that the psychological factors need to be treated and addressed separately from the physical symptoms.

It is of note that perhaps the largest probiotic trial to date by Whorwell et al., whilst showing a significant improvement in Global symptom severity also did not detect any difference in IBS-QOL. [243] Infact, the author of this thesis has been unable to identify any, well designed, probiotic RCT in a western population that utilises the IBS-QOL (English version) that has demonstrated a significant change in this score in the treatment group over placebo.

The use of SSRIs as additional medication at low does was permitted in the study cohort provided that they were started more than 3 months before inclusion in the study and that usage and dose remained constant through the study period. On analysing the data it became apparent that there were considerably more patients taking SSRIs in the probiotic group (13 (10%)) compared to placebo (2 (3%). These patients were not excluded from the analysis but it is possible that the disparity between the number of participants taking SSRIs between the two cohorts may have an influence on the results. Whether taking an SSRI has on affect the likelihood of a placebo response or mute the efficacy of the actual probiotic is not clear. However this difference in the probiotic and placebo cohorts should be noted.

The probiotic bacteria used in this study are presented in the fermentation liquor in which it is produced. This fermentation liquor contains various nutrients and extracts from the germinated barley grain used in the production process of the product. Its reported purpose is to maintain the viability of the bacterial strains within the delivery medium. However, it is

conceivable that as these nutrients act as a growth substrate for the bacterial strains that may also exert a prebiotic effect to other bacterial strains. No previous studies have been conducted to establish whether the probiotic delivery medium itself may be efficacious as a prebiotic in the treatment of IBS but this may warrant further study.

The final point to consider is the observed difference in outcome between males and females in the primary end-point. It has already been discussed that there were significantly more females than males recruited into the study. As this study was only powered to show a change in the overall cohort it would be expected that the gender sub-groups are underpowered. However, this is likely to be more keenly seen in the male sub-group as a result of its considerably smaller size. However, careful inspection of the results from these sub-groups shows that the magnitude of change in the treatment group is similar at -71.24 (SD ± 88.24) and -71.65 (SD ± 89.64) for males and females respectively in the PP analysis and similarly -60.65 (SD ± 84.76) and -59.88 (SD ± 89.48) in the ITT analysis. As can be seen the therapeutic effect is almost identical in the probiotic group for both males and females ($p = 0.98$ (PP) and $p = 0.96$ (ITT)). However, there is a difference in the placebo group with a change of -25.58 (SD ± 86.48) and -37.00 (SD ± 61.65) for females and males in the PP analysis. This observed difference between males and females in the placebo group does not quite reach significance ($p = 0.063$) at the 5% level but a clear trend is seen. It would therefore be reasonable to conclude that the therapeutic effect of the probiotic is observed to be similar in both males and females. Nevertheless,

the smaller sub-group size and apparent increased placebo effect in the male sub-group have resulted in no difference between probiotic and placebo being demonstrated. Based on these observations it is reasonable to suggest that a further study that is sufficiently power may demonstrate a difference between the probiotic and placebo in male patients.

It is important to consider the outcome in this study in the context of comparative studies but, as already discussed, such comparison of IBS clinical trials can be difficult due to a lack of defined end points and considerable heterogeneity of both the condition itself and study methodology. It is perhaps even more important when comparing trials of probiotics as the vast majority of clinical trials and preparations contain different species and strains of probiotic; can contain one or several different probiotic strains; and are presented in different formats (freeze dried capsules, dairy substrates etc). As such, any such comparisons whilst perhaps necessary are likely to be significantly compromised as a direct result of these factors. It is this very conclusion that is reached by the three recent meta-analyses in this subject.[32, 239, 240] Our study is exciting in that it joins one of very few randomised controlled trials into the treatment of IBS with a probiotic preparation that have been conducted using strict and robust research standards, complies to the CONSORT guidelines and have demonstrated clear superiority over placebo.

There has been much discussion and consideration recently of what constitutes a minimal clinically important difference (MCID) in the context of IBS symptoms in clinical trials. For example the creators of the IBS-SSS suggested in their original validation studies that a change of ≥50 points would be clinically significant.[255] In our study the mean change in IBS-SSS in the treatment group was –70.97 (SD ± 88.25) and -63.25 (SD ± 85.95) in the PP and ITT analysis respectively which would indicate an MCID has been observed. However, a later study has suggested that a change of ≥95 points in the IBS-SSS score would represent a MCID.[261] The finding of this later study have not been further corroborated by other research groups and is at odds with the original article. Whilst it is important to comment on this latter article it was not available before the commencement of our study and therefore was not considered its design. As a result our study was not powered to show a change of this magnitude in the IBS-SSS.

In addition, changes in European and American legislation have further tried to clarify MCIDs and specific end-points for clinical trials in the development of new treatments for IBS. The effects of these changes and an apparent increased desired to improve the robustness of IBS clinical trial research is yet to be seen. Our study has demonstrated that this liquid multi-strain probiotic preparation is well tolerated, has a good safety profile and suggests that it is efficacious in the treatment of IBS patients including those with the moderately severe symptoms. Whilst it is of reasonable size and larger that all but a few clinical studies of probiotics in IBS; employs a robust

methodological design and complies with the CONSORT guidelines it remains limited in that it is a single centre study. Furthermore, since its inception, the described changes in opinions and legislation mean that our findings are perhaps already limited in their application. It is therefore essential that further research is undertaken in a large, multicentre randomised controlled trial to not only confirm out original findings but also to update them to meet the latest standards required of research into new therapies for IBS patients.

The findings of our study are exciting and interesting but in many way pose more questions than answers. The efficacy of the studied probiotic in the treatment of IBS is suggested by the results but need be confirmed by a further study. In addition the role of the potential pre-biotic component of the probiotic product should also be considered and further clinical studies may benefit from including a study arm that contains only the delivery medium without the bacterial component. However, given the manufacturing method this may not be achievable. Any further clinical studies should also be adequately powered to allow for sup-group analysis to establish or refute efficacy in both male and female cohorts as well as within IBS disease sub-categories. Finally, and perhaps most importantly, whilst our work proposes the efficacy of the study probiotic in the treatment of IBS it does not give any indication as to the individual characteristics and mechanisms of the included bacterial strains that result in this efficacy. The author considers that further 'basic science' research into the mechanisms of actions of the individual strains of bacteria included in this probiotic are essential to its understanding and potential wider application.

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Appendix 1: Study patient information sheet.

Assessment of Symprove in IBS patients

We would like to invite you to participate in a research trial that we are conducting. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us about anything that
is not clear or if you would like more information. Take your time to decide whether you wish to take part before signing the consent form. Thank you for reading this.

What is the purpose of this study?

The reason for the trial is that some patients such as yourself require specialist treatment for the Irritable Bowel Syndrome (IBS). You have been diagnosed with IBS by your Gastroenterologist. It is likely that you have or are experiencing some of the following symptoms; abdominal bloating and/or pain, intermittent constipation and/or diarrhoea, excessive wind and an alteration in your bowel openings. Some patients also feel tired, experience musclepains (fibromyalgia) or headaches and some women may have symptoms of endometriosis (abdominal pain and irregular periods, etc).
Many treatments are currently available for IBS. Mostly these are dietary (high or low fibre diet, avoidance of dietary products, wheat free diet) or involve medicines such as anti-spasmodic drugs (buscopan, mebeverine, etc.), peppermint preparations and sometimes anti-depressants. The effectiveness of these measures varies greatly and it
is likely that you have tried one or more of these without success. A new approach to the treatment of IBS is increasingly being assessed by Gastroenterologists. This involves giving a mixture of bacteria that are thought to be helpful in maintaining/restoring normal gut function. These bacteria are called pro-biotics but are also commonly referred to as 'friendly bacteria' because of their beneficial effects.

The idea is that the symptoms of IBS may be caused by an interaction between

the bacteria that are found in the intestine and the internal immune system. If, for some reason the normal balance of bacteria changes and there are too many of the "bad" bacteria the symptoms of IBS are thought to emerge. The probiotics are given with a view that they will grow within the gut and restore the normal balance of bacteria, killing off the 'bad' bacteria, reliving symptoms and restoring normal bowel function. As yet
the scientific basis of pro-biotic treatment in IBS has not been tested extensively.

'Symprove' is a liquid containing 3 different "friendly" bacteria. It has been tested in a few patients with IBS and the results are sufficiently promising as for us to conduct a double blind trial on the product to assess how effective it is as compared with placebo treatment (where patients ingest a compound that resembles the Symprove liquid but without the bacteria). These trials are called 'double blind' because neither the researchers or the patient/participant knows who is getting the active product and who is receiving the placebo

Why have I been chosen?

You have been chosen because your Gastroenterologist or General Physician has made the diagnosis of IBS and that you are still symptomatic.

Do I have to take part?

When you are contacted we will describe the study and go through this information sheet answering any questions you have. You will be given a consent form, which should only be signed when you have had time to think about the study and you are sure you want to take part.
You are under no obligation to participate. If you decide to take part you may withdraw from the study at any time and withdrawal will not affect your future treatment in any way.

What will happen if I take part?

We will be contacting persons that have IBS and require treatment. All those that are willing (about 200) to participate will be enrolled into the study. You will be asked to consent to the investigation and the study and your General Physician will be informed about your participation. You will be seen by an experienced research doctor who will document your details and explain the study in detail to you. There will be some extra visits to the hospital and additional blood and faecal samples. You will be asked to rate your symptoms so that we can follow up how these change with the treatment.
When you have consented to participate in the study you will be randomized to having the Symprove or the ”dummy”/placebo preparation. This means that neither you nor your doctor know in which treatment group you are in (although, if your doctor needs to find out he can do so). Two people will receive the active preparation to each on the “dummy”. All the study medication has similar appearance and taste and you will be asked to store the study medication in a fridge and drink (according to your body weight) a fixed amount every day for 3 months. When the study is finished you may want to know the results of the study and if so we will send you a report if you so wish.

What do I need to do?

There is no lifestyle restrictions imposed by this trial and you should go about your life as usual. It is important that you take the study medication regularly as directed and you should continue taking your other regular medication.

What is the preparation that is being tested?

Symprove is a liquid preparation that contains three types of intestinal “friendly” bacteria. These are present in their billions per each millilitre or teaspoon. Symprove and the “dummy” are given on a kilogram basis. For instance if you weigh 60 kg you should take 60 mls of the liquid preparation daily orally.

What alternatives are there for treatment?

This is a study that assesses a new pro-biotic treatment for IBS. Although your consultant Gastroenterologist has requested the treatment there are many treatments for IBS (see above). However many of these are unproven and/or of limited benefit, but you may want to try these rather than the trial medication. Either way you are free to have the conventional treatments and not enter the trial. Patients enrolled in the trial will not be able to use other IBS treatment for the duration of the trial. This will not alter your management in any way in the future.

What are the potential disadvantages and risks of taking part?

There are no disadvantages of participating that we are aware of. The preparation may cause some softening of stools for a few days.

What are the side effects of any treatment when taking part?

Pro-biotics have been shown to be very safe and without major side effects especially in IBS where there is no major damage to the intestine. Symprove is a pro-biotic and we have not come upon any major side effect. A few patients have had loosening of stools for 2-3 days when starting the treatment. Other potential side effects include diarrhoea and excessive wind, but these are infrequent and mild.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study will help the treatment of people with IBS.

What if new information becomes available?

Sometimes during the course of research projects, new information becomes available about the condition that is being studied. If this happens, your doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

What happens when the research study stops?

In the unlikely event that the study stops before we have recruited all the patients that we require you will be informed that your participation is not required

What if something goes wrong?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Compensation for any injury caused by taking part of this study will be in accordance with the guidelines of the Association of the British Pharmaceutical Industry (ABPI). Broadly speaking the ABPI guidelines recommend that "the sponsor", without legal commitment, should compensate you without you having to prove that it is at fault. This applies in cases where it is likely that such injury results from giving any new drug or any other procedure carried out in accordance with the protocol for the study. "The sponsor" will not compensate you where such injury results from any procedure carried out which is not in accordance with the protocol for the study. Your right at law to claim compensation for injury where you can prove negligence is not affected.
Copies of these guidelines are available on request.

Complaints about the study.

If you have concerns about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Dr Guy Sisson 07958342353). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Will my taking part in this study be kept confidential?

Yes. Data about this research will be secured against unauthorised access and no individual will be identifiable from published results without their prior consent. If, exceptionally, we wish to retain confidential information beyond completion of the research project, we will undertake to let you know the reasons for retaining the information and the circumstances in which this might be disclosed. In view of this, we need your consent to these arrangements.

What will happen to the results of the research study?

The results will be analysed and published in a scientific journal. If you so wish we can send you a copy when available.

Who is organising and funding the research?

This research has been instigated by Professor Bjarnason and is supported by the inventors of Symprove namely Symprove Ltd. That is based in Guilford.

Who has reviewed this study?

The Bromley Research Ethics Committee has reviewed these studies (not "approved").

You will be given a copy of the information sheet and a signed consent form to keep.

We do hope you will be able to participate. If you have any further questions please contact Professor Ingvar Bjarnason on 020-3299-3417 or 07784589003. If you do take part of this study and need immediate advise you can contact Dr Guy Sisson during daytime and 07958342353 out of hours.

Yours sincerely

Professor Ingvar Bjarnason
Professor of Digestive Diseases
Consultant Physician and Gastroenterologist

Appendix 2: Study patient consent form

CONSENT FORM

Centre number: …….
Study number: …….
Patient Identification number
for this trial: …….

Title of Project: Assessment of Symprove in IBS patients

Name of Researcher: Prof. I Bjarnason (telephone 07784589003 at any time)

Please initialise box

I confirm that I have read and understood the information sheet dated June 2008for the above study and have had the opportunity to ask questions ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected ☐

I understand that sections of any of my medical notes may be looked at by responsible individuals from Symprove Ltd or from regulatory authorities were it relevant to my taking part in research. I give permission for these individuals to have access to my records ☐

I agree to take part in the above study

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	
Name of Person taking consent	Date	Signature
_____	_____	
Researcher	Date	Signature

Three copies will be made for: (1) Patient, (2) Researcher, (3) Hospital

Appendix 3: Illustration of Randomisation protocol.

Randomisation protocol illustration

A		B		C		D	
Group number	Group number randomised (Column 2)	Trial ID number	Trial ID number Randomised (Column 4)	Group No. Randomised (column 2)	Trial ID number randomised (column 4)	Group no. allocation in ascending order of trial ID number	Ascending Trial ID No.
1	2	1	13	2	13	2	1
1	1	2	6	1	6	3	2
1	1	3	11	1	11	3	3
1	3	4	18	3	18	2	4
1	2	5	4	2	4	2	5
1	2	6	5	2	5	1	6
1	3	7	21	3	21	1	7
2	1	8	17	1	17	1	8
2	3	9	2	3	2	1	9
2	1	10	8	1	8	3	9
2	2	11	12	2	12	1	11
2	3	12	3	3	3	2	12
2	2	13	16	2	16	2	13
2	3	14	20	3	20	2	14
3	2	15	14	2	14	3	15
3	2	16	1	2	1	2	16
3	1	17	9	1	9	1	17
3	1	18	19	1	19	3	18
3	1	18	7	1	7	1	19
3	3	20	9	3	9	3	20
3	3	21	15	3	15	3	21

Illustrations of Randomisation using the Mersenne twister algorithm

A: Randomisation of group number (column 2) (Stage 1 randomisation)

B: Randomisation of trial ID number (column 4) (stage 2 randomisation)

C: Combining of randomisations of group number and ID number (columns 2+4)

D: Resorting of C in ascending order of trial ID number.

Appendix 4: Questionnaire 1: IBS-SSS

QUESTIONNAIRE 1

(IBS-SSS)

WEEK: _____ (Date____/____/20____)

PATIENT DETAILS:

NAME: _____

dob ____/____/19____

STUDY ID No: _____ Hospital No: _____

INSTRUCTIONS

1. For questions where a number of different responses are a possibility then please circle the response most appropriate to you.

2. Some Questions ask you to write a response.

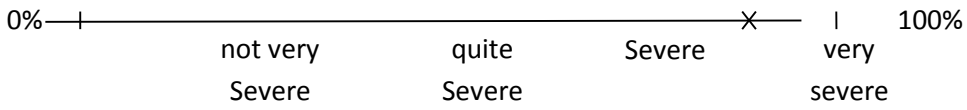
3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:

How severe is your pain?

Please indicate with a (X) anywhere on the line between 0-100% in order to indicate as accurately a possible the severity of your symptoms.

This example shows a severity of approximately 90%



ALL INFORMATION OBTAINED DURING THE STUDY, INCLUDING YOUR RESPONSES TO THE QUESTIONNAIRES WILL BE KEPT STRICTLY CONFIDENTIAL

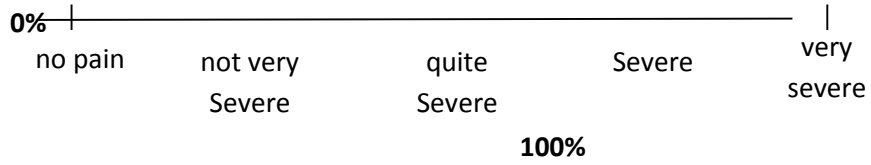
Please fill in the date you completed the Questionnaire:_____

PART 2: SYMPTOM SEVERITY SCORE

1, a) Do you currently suffer from abdominal (tummy) pain? YES | NO

circle appropriate box

b) If yes, how severe is your abdominal (tummy) pain?



c) Please enter the number of days that you have had pain over the last 10 days.

For example if you enter 4 days it means you had pain 4 out of the last 10 days. If

you get pain every day enter 10.

Number of days with pain ☐

x10

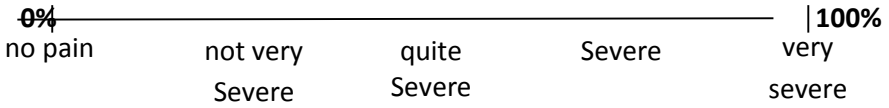
2, a) Do you currently suffer from abdominal distension?*

(bloating/swollen or tight tummy) YES | NO

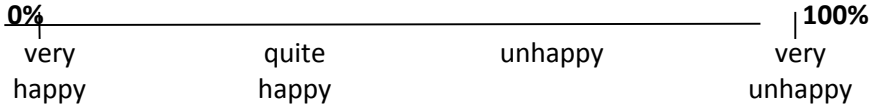
circle appropriate box

(*women, please ignore distension related to your periods)

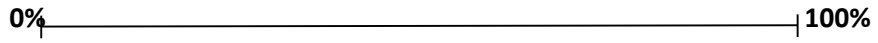
b) If yes, how severe is your abdominal distension/tightness



3, How satisfied are you with your bowel habit?



4, Please indicate with a cross on the line below how much your irritable bowel syndrome is affecting or interfering with your quality of life in general



not at all | not much | quite a lot | completely

IBS SEVERITY SCORE

IBS QUESTIONNAIRE

PART 2: OTHER IBS DATA BOWEL HABIT

5, a) What is the most number of times you open your bowels per /day/week/month?

Number of times
per day/week/month (circle appropriate)

Note: For some people the answers to part a and b could be the same

b) What is the least number of times you open your bowels per day/week/month

Number of times
per day/week/month (circle appropriate)

6. In the following questions you may circle more than one answer:

Are your motions ever:

a) normal **often/occasionally/never** (circle appropriately)
b) hard **often/occasionally/never** (circle appropriately)
c) very thin (like string) **often/occasionally/never** (circle appropriately)
d) In small pieces **often/occasionally/never** (circle appropriately)
(like rabbit pellets)
e) mushy (like porridge) **often/occasionally/never** (circle appropriately)
f) watery **often/occasionally/never** (circle appropriately)

7. In the following questions you may circle more than one answer:

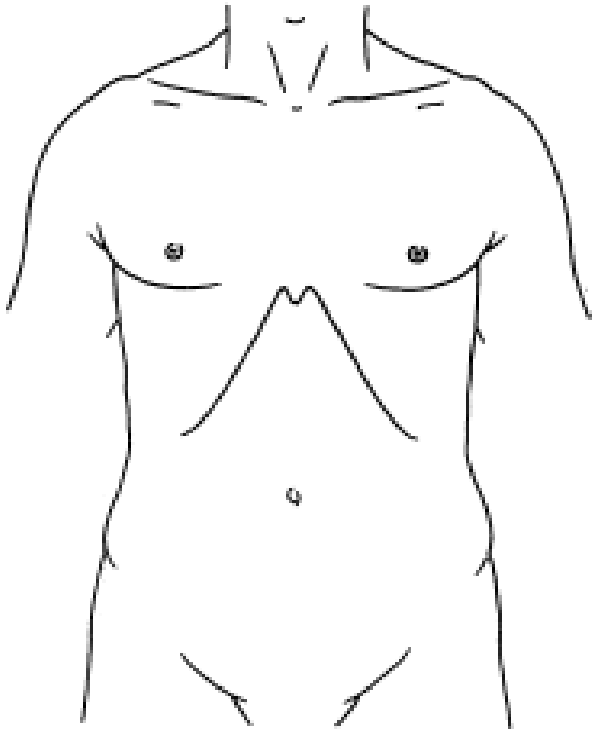
Do you ever:	Circle box appropriately	
a) pass mucus (or slime or jelly) with your motions	**YES**	**NO**
b) pass blood with your motions	**YES**	**NO**
a) have to hurry/rush to the toilet to open your bowels	**YES**	**NO**

b)	**strain to open your bowels**	**YES** / **NO**
c)	**feel you haven't emptied your bowel completely after you have passed a motion**	**YES** / **NO**

IBS QUESTIONNAIRE

SITE OF PAIN

Please mark with a cross (x) on the diagram below where you get your pain
(use more than one cross if necessary)



8. **Do you ever:**

a) **Notice your stools are more frequent of loose when you get pain** — **YES** | **NO** — Circle box appropriately

b) **notice whether your pain is frequently eased by opening your bowels** — **YES** | **NO** — Circle box appropriately

9. **In the last year on approximately how many weeks were you:**

a) **absent from work due to IBS** ☐

(enter 52 if you have given up work completely due to IBS)

b) at work suffering from IBS ☐

Appendix 5: Questionnaire 2: IBS-QOL

PLEASE WRITE IN
TODAY'S DATE: _____ _____ _____
DAY MONTH YEAR

PARTICIPANT/PATIENT ID:

PLEASE READ THIS CAREFULLY

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (IRRITABLE BOWEL SYNDROME) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE CHOOSE THE RESPONSE THAT BEST APPLIES TO YOU AND **CIRCLE** THE NUMBER OF YOUR RESPONSE.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT, PLEASE GIVE THE BEST RESPONSE YOU CAN. **THERE ARE NO RIGHT OR WRONG RESPONSES.**

YOUR RESPONSES WILL BE KEPT STRICTLY CONFIDENTIAL.

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT:

About Your Feelings

Please think about your life over the **past month (last 30 days)** and look at the statements below. Each statement has five possible responses. For each statement, please circle the one response that best describes your feelings.

1. I feel helpless because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

2. I am embarrassed by the smell caused by my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

3. I am bothered by how much time I spend on the toilet. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 A GREAT DEAL

4. I feel vulnerable to other illnesses because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

5. I feel fat or bloated because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 A GREAT DEAL

6. I feel as though I am losing control of my life because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

7. I feel that my life is less enjoyable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

8. I feel uncomfortable when I talk about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

9. I feel depressed about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

10. I feel isolated from other people because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

11. I have to be careful about the amount of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

12. Because of my bowel problems sexual activity is difficult for me. *(Please circle one number)*
(If not applicable, please circle "NOT AT ALL")

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

13. I feel angry that I have bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

14. I feel as though I irritate others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

15. I worry that my bowel problems will get worse. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

16. I feel irritable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

17. I worry that people think I exaggerate my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

18. I feel that I get less done because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

19. I have to avoid stressful situations because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

20. My bowel problems reduce my sexual desire. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

21. My bowel problems limit what I can wear. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

22. I have to avoid strenuous activity because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

23. I have to be careful about the kind of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

24. Because of my bowel problems I have difficulty being with unfamiliar people. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

25. I feel sluggish because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

26. I feel “unclean” because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

27. Long trips are difficult for me because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

28. I feel frustrated that I cannot eat when I want to because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

29. It is important to be near a toilet because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

30. My life revolves around my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

31. I worry about losing control of my bowels. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

32. I am afraid that I won't be able to have a bowel movement. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

33. My bowel problems are affecting my closest relationships. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

34. I feel that no one understands my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY